ABSTRACT OF THESIS

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Title of Thesis "Analytical and Structural Studies in the Polysaccharide Group"

The studies reported in this thesis fall into three parts:-

Part 1. Introductory review of the analytical methods currently available for analytical and structural studies of polysaccharides, together with a brief review of the present state of knowledge of gum exudates of the Acacia family.

Part 2. Comparative Analytical Studies.

Comparative analytical studies were carried out on:-

(a) sixteen samples of A.senegal gum differing in origin. Only slight differences in the overall composition and physical properties of both the crude and purified gum were observed.

(b) seven different, previously unstudied, species of Acacia gum. Marked variations in both composition and properties were observed from species to species, which were studied in both the crude and purified forms.

(c) ten specimens of the gum from A.nilotica were analysed to assess the extent to which inter-nodule variation occurred.

Fractionation of this gum was attempted on DEAE-cellulose. One major fraction and several minor fractions were obtained; on preliminary analysis, the major fraction did not differ significantly from the 'parent' gum.

Part 3. Structural Studies.

Detailed structural studies were carried out on a typical sample of A.nilotica gum.

A.nilotica gum ($[\alpha]_D^{20}$ + 108°) contains residues of D-galactose (44%), L-arabinose (46%), L-rhamnose (0.4%), D-glucuronic and 4-O-methyl-D-glucuronic acid (totalling 9%).

Partial acid hydrolysis gave a mixture of neutral and acidic disaccharides of which the following were isolated and identified:-

3-O-β-L-arabinopyranosyl-L-arabinose
3-O-β-D-galactopyranosyl-D-galactose
6-O-β-D-galactopyranosyl-D-galactose
6-O-(β-D-glucopyranosyluronic acid)-D-galactose
6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose
4-O-(α-D-glucopyranosyluronic acid)-D-galactose
4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose.

Hydrolysis /

Use other side if necessary.

Hydrolysis of methylated gum afforded:

2,3,5-tri-O-methyl-L-arabinose
2,3,4-tri-O-methyl-L-arabinose
2,5-di-O-methyl-L-arabinose
2,3-di-O-methyl-L-arabinose
2,3,4,6-tetra-O-methyl-D-galactose
2,3,4-tri-O-methyl-D-galactose
2,4,6-tri-O-methyl-D-galactose
2,4-di-O-methyl-D-galactose
2,3,4-tri-O-methyl-D-glucuronic acid.

Smith degradation was carried out on the degraded gum, and the results confirmed the presence of both 1,6 and 1,3 linkages in the galactan framework of the gum molecule.

The structural features of this gum have been summarised and discussed in the light of these results.

ANALYTICAL AND STRUCTURAL STUDIES IN THE POLYSACCHARIDE GROUP

BY

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THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF THE UNIVERSITY OF EDINBURGH IN THE FACULTY OF SCIENCE

June, 1965

TO MY COUNTRY

I N D E X

PART I

INTRODUCTION AND EXPERIMENTAL

GENERAL INTRODUCTION

Plant gums are complex polysaccharides of high molecular weight and in general they are built up by the union of sugar residues of several different kinds. For instance, the molecule of gum arabic contains D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid (1). Other sugar residues known to occur in plant gums are D-xylose, D-mannose and L-fucose, while acetylated and methylated sugar residues are not uncommon (2).

Plant gums are generally formed as exudates to cover wounds on the bark or fruit of trees in order, it is believed, to prevent attack by micro-organisms. Shortly after injury, a viscous fluid appears on the surface of the plant at the point of injury, and this gradually dries to a hard glassy nodule. Plant gums are colloidal and find extensive application in industry as sizes, particularly in the textile and printing industries, as adhesives, and as binding agents in the pharmaceutical trade. The largest consumer of gums, however, are probably the confectionery and food-stuffs industries.

Although more than 500 species of *Acacia* are known botanically (3), commercial *Acacia* gum, produced largely in the Sudan and Nigeria, is produced essentially from one of these species, *Acacia senegal* (syn. A. verek). A number of other species of *Acacia* are exploited to a limited extent, e.g. *A.seyal* and *A. drepanolobium*. The Sudan has 22 indigenous *Acacia* species which include, in addition

to the above three, A. arabica, A. nilotica, A. nubica, A. campylacantha, A. dealbata, A. fistula and A. laeta (4). Restriction in the collection of gum to A. senegal has led to a more uniform product, and adulteration is reduced. This species can grow on both sandy and clay soil in the semi-desert, reaching a height of 15 to 20 feet. A. nilotica, which grows to a height of about 30 feet, is found mainly along the Nile banks in the central Sudan. It is not normally tapped but gum is obtained from it as a natural exudate. This species has long spines and a dark bark, and constricted seed pods which are used for tanning and medical purposes; its wood is used for making railway-line "sleepers".

PURIFICATION AND FRACTIONATION

Before starting the investigation of a gum, an enquiry should be made as to its origin. Wherever possible, specimens which have been collected by a reliable botanical or chemical expert are preferable to samples from commercial sources. Commercial gum arabic is known to contain small percentage of nodules of the gum from A. laeta as this tree grows in the "pockets" among A. senegal stands and its gum is visually indistinguishable from that obtained from A. senegal.

The acidic polysaccharides in the crude gum usually exist in the form of neutral or slightly acidic salts, with calcium, magnesium, iron, sodium, and potassium usually present as cations. Purification is frequently carried out by dissolving the gum in water, followed by precipitation from the filtered solution with an organic reagent; generally, acidified ethanol is used.

Although it has been reported that the ash content of some species of gum can be eliminated (5, 6) by precipitation methods, experiments (7) with Acacia species have shown that their ash content cannot be reduced by more than about 50%, even after four precipitations. Electrodialysis, in our experience, is the most effective method of reducing the ash content to a very low level. In addition, gums, particularly Acacia gums, contain a small percentage of nitrogen, which is not reduced by any method of purification. As the mechanism of gum formation is still far from clear (8), further knowledge of the nature of this nitrogen content in plant gums would be of value. It might then be possible to assess the the relative importance of the enzymic polymerisation theory (3) in relation to the alternative theories (8, 9) that gum formation results from (a) normal plant metabolism or (b) pathological reactions to resist the invading micro-organisms or to avoid excessive loss of moisture (3).

If a gum is only slightly soluble in water, alkali can be used to effect dissolution, but great care should be taken in controlling the reaction conditions, since the alkali may degrade the polysaccharide (10, 11, 12).

The precipitated or electrodialysed polysaccharide material cannot be assumed to be homogeneous, since evidence of heterogeneity has been noted in the case of several gums. Gum tragacanth has been shown to consist of a mixture of polyglycosidouronic acid and a galactoaraban (13). Gum arabic and the gum obtained from A. seyal are known to vary in their rhamnose content (14, 7). In such cases it is not possible to put forward a unique molecular

structure, but it is legitimate to indicate general features. The main fractionation techniques available for the examination of polysaccharides include fractional precipitation, complex formation, preferential solubility, various chromatographic methods, and electrophoresis.

Fractional precipitation is the simplest method. It involves the addition of a precipitant to an aqueous solution of the gum and the subsequent isolation of fractions or components having different solubilities. Co-precipitation may occur, and may necessitate several reprecipitation of each component. The gum components of Olibanum (15), and Khaya senegalensis (16) have been fractionated in this manner, the former into a neutral and an acidic component, and the latter into two acidic polysaccharides of high and low uronic acid content.

Another technique now available for fractionating polysaccharide mixtures makes use of the fact that some polysaccharides form insoluble salts or complexes with certain reagents. The commonest of the complexing agents are cupric acetate (17), Fehling's solution, and long chain quarternary ammonium salts, such as cetyltrimethylammonium bromide ("Cetavlon"). The latter gives precipitates with many acidic polysaccharides (18, 19) and may be used to separate them from non-acidic components (20). In this laboratory (21) fractionation of A. senegal gum into polysaccharides differing considerably in their molecular weights and nitrogen content, has been effected by means of precipitation from aqueous solution with sodium sulphate.

Gum tragacanth, which consists of an acidic polysaccharide, an arabogalactan and a glycoside, was fractionated (13) by

differences in the solubilities of the methylated components.

The fractions obtained by fractional precipitation and complex formation can then be examined for homogeneity by means of chromatography, gel-filtration or electrophoresis. The first two of these techniques have been used on a preparative scale.

The separation of polysaccharide mixtures by chromatography has recently taken great steps forward with the introduction of ion-exchange celluloses such as DEAE-cellulose (22) and ECTEOLA-cellulose (23). Anion exchange cellulose, in different forms (phosphate, borate, etc.) retains acidic polysaccharides at neutral pH values, whereas neutral polysaccharides are not or are only weakly absorbed. Thus by using a suitable elution medium at different pH values and electrolytic concentrations, various polysaccharide mixtures have been fractionated (16).

Separation of polysaccharides can be effected by passage through a column of cross-linked dextran, known as "Sephadex". A clear-cut separation of some acid mucopolysaccharides has been obtained by ion-exchange chromatography on DEAE-Sephadex (24). "Gel-filtration" is proving useful in estimating molecular weights of fractions of A. senegal obtained by fractional precipitation with salts (21).

The main advantage of electrophoresis in assessing polymer homogeneity is its speed, and also the fact that very little material is required. Using this technique, Lewis and Smith (25) examined a number of gums, and as well as confirming heterogeneity in gum tragacanth and in gum arabic, they found two components in gum ghatti and A. pycnantha ; the latter two gums had previously

been thought to be homogeneous.

The different types of heterogeneity which occur in gums range from the kind in which the constituent polysaccharides contain slightly different proportions of certain sugars to the kind of heterogeneity in which the component polysaccharides are of completely different polymeric types. Anderson, Hirst and King (26), in a detailed examination of several nodules of Combretum leonense gum, found significant differences in the proportions of sugar residues in the various samples. This internodule variation was also observed in the investigation of A.seyal (Del.) (7). As has been mentioned earlier, two polysaccharide fractions have been isolated from Khaya senegalensis (16) and these were shown to be of entirely different types. Fractionation of gum tragacanth (13) furnished two structurally unrelated polysaccharides. In contrast, heterogeneity due solely to differences in molecular size has been observed by fractional precipitation of A. senegal with sodium sulphate; the molecular weights of the fractions, as found by "gel-filtration" on Bio-Gel P300, ranged from about 100,000 to one million (21).

So in view of the evidence of the widespread heterogeneity in gums, the important question arises of whether a definite structure , characteristic of the species, can be assigned to any gum. It seems likely, however, that, whilst a few gums, such as gum tragacanth,contain widely differing polysaccharides, the majority of gums may consist of 'families' of closely similar polysaccharides. These may, for example, possess a 'back-bone' of constant structure, to which variable side chains are attached. Although the extent of the variation is probably slight for any

one species, however, it appears to increase considerably for different species within the genus as a whole so that, generally, only broad structural characeristics are shared.

GENERAL METHODS EMPLOYED IN STRUCTURAL INVESTIGATIONS OF PLANT GUM POLYSACCHARIDES:

In order to obtain a general picture of the polysaccharide, some preliminary analyses are carried out. These include the determination of (a) its physical constants (such as ~~optical~~ specific rotation, neutralisation equivalent, and viscosity coefficients) and (b) its chemical composition (e.g. its hexose, pentose, uronic anhydride, acetyl, methoxyl, nitrogen and ash contents). Total acid hydrolysis is used to determine the nature and relative proportions of the monosaccharide residues present. Separation is usually achieved by column or paper chromatography. Quantitative estimation of sugar residues can be carried out rapidly on a micro-scale by paper chromatography (27) in conjunction with some micro-analytical technique for estimating sugar concentrations, e.g. the phenol-sulphuric acid method,(28) and the Somogyi method (94). Incomplete breakdown of the polymer and degradation of the monosaccharides liberated may give rise to errors; usually a determination of the absolute amounts of the monosaccharide constituents of the polymer is achieved by including, as a reference sugar, a monosaccharide which is known to be absent in the polymer.

Probably the most successful general methods available for the detailed structural studies of polysaccharides are partial acid hydrolysis, methylation, and periodate oxidation. Partial

hydrolysis is used to determine some of the sequences of monosaccharide constituents, by the isolation of oligosaccharides formed during the breakdown of the polysaccharide molecule. Information concerning the mode of linkage of each sugar unit, the proportion of non-reducing end-groups, and, in some cases, the ring structure of the sugar is achieved by methylation procedures. Oxidation of the polysaccharide with periodate yields a polysaccharide of lower molecular weight, which, as observed below, can provide information as to the structure of the original polysaccharide.

PARTIAL ACID HYDROLYSIS

Partial hydrolysis is by far the most useful technique employed in the extremely complex problem of determining the sequence of sugar units in a polysaccharide. This involves the characterisation of di- and oligosaccharides which have resulted from the incomplete breakdown of the polymeric molecule.

The stability of the glycosidic links towards acids varies considerably according to the class of the sugar, and to the ring form. Furanosides are in general much more acid-labile than pyranosides; in fact the acidity of a hot aqueous solution of a gum may be sufficient to strip off arabinose end-groups, which usually exist in the furanoside form. This process, known as autohydrolysis, produces a degraded polysaccharide having a simpler structure than the original gum. The use of very weak mineral acids in aqueous solutions will generally give reasonable yields of neutral di- and higher oligosaccharides. Aldobiouronic acids may be isolated after hydrolysis of the polysaccharide

under more vigourous conditions.

It is often difficult to determine the nature of the most acid-labile linkages, since these will be the first to be cleaved on acid hydrolysis and will therefore not be present in any of the oligosaccharides. In such cases resort is made to acetolysis. Thus the acid-labile rhamnose residues in Acacia senegal have been shown to be attached to position 4 of the glucuronic acid residue by isolation of the oligosaccharide 4-O-L-rhamnopyranosyl-D-glucose from partial acetolysis products of the reduced gum (29).

Another technique which may prove useful in the elucidation of structures is that of catalytic oxidation. By this means, it is possible to oxidise selectively the primary alcohol groups in a polysaccharide to carboxyl groups. This provides a method for determining the nature of linkages which involve acid-labile sugar residues, such as arabofuranose end-groups. Under the circumstances, it is impossible to isolate the disaccharides concerned by partial hydrolysis. The corresponding aldobiouronic acid, however, is easily isolated from the oxidised polysaccharide, since the glycuronosyl link is acid-resistant, and its identification indicates the nature of the original glycosidic linkage (30).

Enzymolysis (enzymic hydrolysis) in comparison with acid hydrolysis has the advantage of specificity. When the 1,4-linked unbranched glucan, amylose, is hydrolysed by meanes of β-amylase, fragmentation occurs in a systematic fashion to yield maltose as the only final product. In contrast, acid hydrolysis cleaves glycosidic linkages at random. Whereas enzymolysis has been

used with success in studies of some homopolysaccharides, the use of enzymes has been limited in the case of heteropolysaccharides which contain a diversity of building units and linkages.

The structural significance of oligosaccharides present in trace quantities in hydrolysis products may be doubtful, because it has been shown that, on heating a solution of monosaccharides with acid, oligosaccharides are formed (31, 32) as "reversion" products". However, these can be distinguished since they reach an equilibrium concentration and do not disappear on prolonged heating (33). The difficulty which arises through the acidic decomposition of the oligosaccharides liberated as the reaction proceeds may be overcome by carrying out the hydrolysis in a dialysis bag, thus continuously removing the low molecular weight material formed (34). This method was first used for autohydrolysis, but by using a water-soluble acidic resin (35) as the hydrolytic agent, its scope can be greatly increased.

The mixture of oligosaccharides can be separated from monosaccharides and from one another by chromatography on charcoal-celite (36), cellulose (37), or resin (38) columns, or by thick paper chromatography. Aldobiouronic acids can be successfully separated on weakly basic anion-exchange resin columns (39). Several disaccharides and their derivatives are themselves crystalline and so can be identified directly. The component sugars of the oligosaccharides are identified by hydrolysis, or by reduction followed by hydrolysis if it is an acidic oligosaccharide. An indication of the identity of an oligosaccharide can be obtained from its chromatographic mobility in various solvents, ionophoretic behaviour and ~~optical~~ specific rotation. The mode of

linkage is established by methylation, followed by hydrolysis and characterisation of the demethylated derivatives. Identification of the oligosaccharides can be carried out on a micro-scale by complete methylation and subsequent examination of the methanolysis products by gas/liquid chromatography (40). Periodate oxidation (41) can give useful information regarding the type of linkage and the nature of an oligosaccharide.

PERIODATE OXIDATION

The ability of the periodate ion to cleave the carbon-carbon bond in $\alpha\beta$-glycols (42, 43) is used in structural studies on polysaccharides. In most instances the arrangement of the diol or triol groups may be deduced from measurements of the consumption of oxidant and determinations of the nature of the oxidation products (formaldehyde, formic acid, carbon dioxide).

The periodate oxidation is influenced by temperature, pH, concentration of reactants and light, and in general the reaction conditions are carefully regulated to avoid "over oxidation" (44).

When three carbon atoms α,β,γ are linked together and each has a free hydroxyl group, the $\alpha\beta$ and $\beta\gamma$ bonds are broken with the formation of one molecule of formic acid. Estimation of the formic acid released will therefore afford a measure of the frequency with which such a structural feature occurs. Only terminal sugar residues and 1,6-linked hexopyranose residues may possess three adjacent hydroxyl groups, and it may therefore be possible to assess the degree of branching in certain polysaccharides. Since formaldehyde may arise from the terminal residue at the reducing end of the polysaccharide, this fact has been used to

estimate molecular weights. Also the determinations of formaldehyde after periodate oxidation is reported to distinguish between 1:4 and 1:6 linked disaccharides (45).

Since one molecule of oxidant is consumed for each $\alpha\beta$-glycol group cleaved, it is possible, therefore, by estimating the amount of periodate reduced, to distinguish between certain alternative structures. If a linear polysaccharide is known to consist of hexopyranose residues linked through their 1,4-and 1,6-positions only, the proportion of each may be calculated from the periodate uptake because they consume one and two molecules of oxidant respectively(46). The periodate is usuallyestimated by a standard arsenite solution (47) or by a spectrophotometric method (48).

Advantage is frequently taken of the fact that, in the application of periodate oxidation to polysaccharides, some of the sugar units are not susceptible to attack by the reagent since they do not possess adjacent hydroxyl groups. Thus for hetero-polysaccharides such as gums the unoxidisable residues of each type of monosaccharide can be characterised and their relative proportions estimated (49). An improvement on this method is the selective removal of the fragments of cleaved residues, thus leaving the unoxidised residues open to further oxidation. In the Barry degradation (50) the polyaldehyde is treated with phenyl-hydrazine and the fragments removed as the phenylosazones. Using this procedure, gum arabic was found to have a 'back-bone' of 1,3-linked galactose units (49).

A modified periodate degradation procedure is based on the mild acid hydrolysis of the polyalcohol obtained after borohydride

reduction of the periodate-oxidised polysaccharide and is known as the Smith degradation (51,52). The conditions of hydrolysis (usually at room temperature with dilute mineral acid) are such that the acetal systems are cleaved whereas glycosidic bonds are stable. If any polysaccharide remains after this degradation it is separated from the rest of the products by precipitation with an organic solvent and treated again with periodate or subjected to partial hydrolysis, methylation, or other means of structural investigation. By carrying out a Smith degradation on degraded gum arabic, a linear 1,3-linked galactose was obtained (53) similar to the one obtained by Dillon from the Barry degradation of arabic acid.

Difficulties, however, are encountered when applying the Smith degradation to acidic polysaccharides since the oxidised fragments which contain uronic acid groups are less readily cleaved by mild acid hydrolysis than the other acetal systems. One way of dealing with this difficulty is to reduce the uronic acid residues before commencing the oxidation. Satisfactory results have been obtained by reducing the fully acetylated polysaccharide with diborane (54), but as side effects are known to arise in diborane reductions, such results must be considered with caution.

METHYLATION STUDIES:

The classical method of methylation, followed by hydrolysis and identification of the hydrolysis products, is probably still the most useful method of structural investigation. The methoxyl group introduced is chemically stable.

The principle underlying this method depends on the complete methylation of all hydroxyl groups not involved in glycosidic linkages. Hence on hydrolysis of the resulting methylated polysaccharide, the methyl sugars obtained will carry hydroxyl groups only on those carbon atoms which were involved in glycosidic linkages or ring formation. Identification of these partially methylated sugars will then provide evidence of the nature of the sugar residues, and of their modes of linkage in the polysaccharide if the ring size of the sugars residue is known. The methylation results may also indicate the non-reducing terminal residues per average building unit. Certain significant structural features become apparent if results are available from methylation studies alone on the degraded and undegraded gum e.g. mesquite gum, despite the complexity of the polysaccharide (8). However, methylation studies become more diagonostic when considered, as is usualy in conjunction with periodate oxidation studies and controlled degradation.

The standard method of methylation using dimethyl sulphate and 30% sodium hydroxide was developed by Haworth (55). As this method rarely gives full methylation, especially with acidic polysaccharides, methylation may be completed by Purdie's method (56) using methyl iodide and silver oxide, or Kuhn's method (57) which uses barium oxide and methyl iodide in N,N-dimethyl formamide as solvent. A recent modification of Kuhn's method uses dimethyl sulphoxide (DMSO) (58) as solvent. Although this method gave theoretical yields with neutral polysaccharides, it proved less successful when applied to acidic polysaccharides like the Acacia gums.

Other less commonly used methods available for methylation make use of thallium hydroxide and methyl iodide (59), diazomethane (60), and methyl iodide with sodium in liquid ammonia (61, 62).

The fully methylated polysaccharides are insoluble in hot inorganic acids and therefore cannot be directly hydrolysed. This difficulty may be overcome by preliminary methanolysis (63) or formolysis (64) followed by hydrolysis. Methylated acidic sugars can be separated from the methylated neutral sugars by absorption on ion-exchange resins (65). The products of hydrolysis can be separated by chromatography on cellulose columns and the individual methylated sugars characterised by means of crystalline derivatives. Since certain methylated sugars are reported to be volatile (66), the relative proportions of methylated sugars from the methylation studies of a particular polysaccharide should be regarded with caution.

Recently, the use of gas/liquid partition chromatography for the separation of methylated and partly methylated methyl glycosides has been reported by Bishop (67). This provides a highly selective method for the analysis of the cleavage products from methylated oligo- and polysaccharides. The relative retention times of a large number of methyl glycosides are now available for reference purposes (68).

MOLECULAR STRUCTURE OF ACACIA GUMS

To date, the gums from eight species of the genus Acacia (family leguminoseae) have been studied; these include A. senegal (69, 70, 71), A. pycnantha (72, 73), A. cyanophylla (74), A. karroo (75), A. mollissima (76), A. sundra (77), A. catechu (78) and A. seyal Del (79); preliminary investigations have been carried out on seven more Acacia gums of botanical authenticity and the results of these studies are presented in this thesis together with a detailed structural study of one of these gums. Comparison of the composition and properties of Acacia gums studied so far, reveals that they all contain the same sugar residues, mamely, D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid (Table II). However, the proportions of these components are markedly different, and the specific rotations of the gums differ widely also. These features must reflect differences in the fine structures of the individual gums. In particular the proportion of uronic acid in the gums differs considerably and consequently, if the general structural features of the Acacia gums are the same, it would appear that they may differ in the ratio of acidic side chains to D-galactose residues in the backbone. For example, for A. senegal this ratio is 3:6 for A. cyanophylla 5:6, for A. mollissima 1:4, and for A. pycnantha 1:2. These ratios may be significant in comparing the physical properties of the respective gums (80).

To date, however, the possibility of the presence of methoxyl groups in Acacia gum exudates appears largely to have been ignored although Stephen reported (without comment) a value of 0.35% for A. mollissima (81), and Hulyalkar et.al. found no methoxyl content in A. catechu (82). Recently Anderson et. al.

(83) have shown that the presence of methoxyl groups in Acacia gums is a more general occurrence than hitherto believed: the methoxyl contents of 15 Acacia species range from 0.13% (A. karroo) to 2.4% (A. giraffae Birch) and a plot of methoxyl contents versus limiting flow-time number gives a smooth curve. The suggestion that the methoxyl content of Acacia gums may have some structural significance is borne out by the isolation of the substituted aldobiouronic acids in the detailed structural studies of A. nilotica (average % OMe = 1.14) undertaken in the present work (Table III). Re-examination of the acidic fractions from the partial acid hydrolysis from A. senegal and A. seyal and others now in progress now in this laboratory (84), indicates the presence of aldobiouronic acids containing 4-O-methyl-D-glucuronic acid.

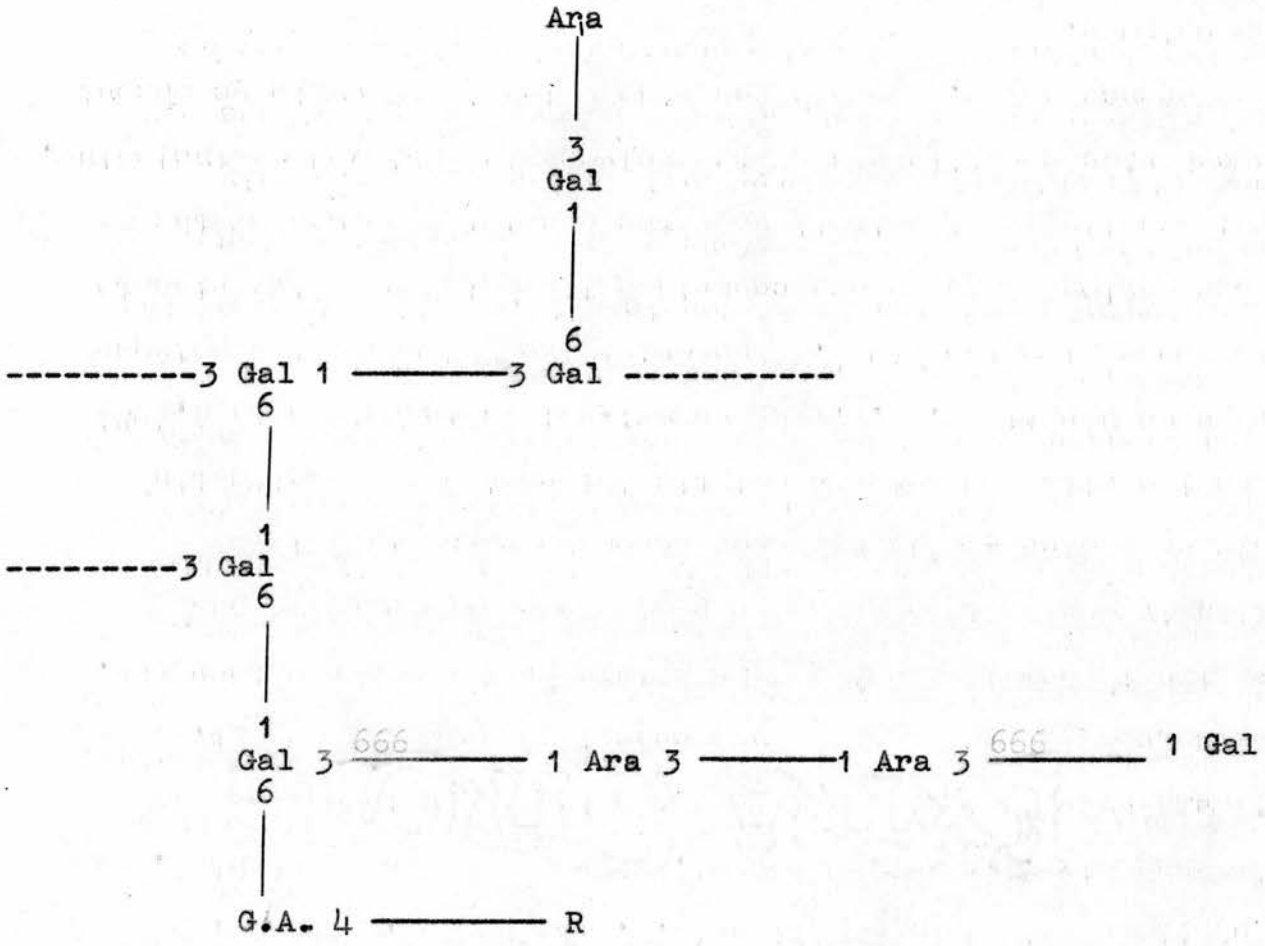
Acacia gums undergo autohydrolysis to give L-arabinose and neutral disaccharides, and a degraded gum which contains essentially, D-galactose and D-glucuronic acid. Some rhamnose may also be liberated, but is ~~is~~ generally less readily removed than arabinose. The acidic residues may occur in the labile side chains as well as in the more resistant core of the degraded gum, evidence to this effect having been found for the gums from A. karroo and A. cyanophylla (75,74). Recently also Aspinall (85) has reported the isolation of 4-O-L-rhamnose-D-glucose from A. senegal after a series of reaction involving acetylation and reduction, thus giving support to the theory of the existence of D-glucuronic acid residues in the periphery of the structure.

The same aldobiouronic acid, 6-O-β-D-glucuronosyl-D-galactose has been detected in all members so far examined, but A. karroo yields in addition another aldobiouronic acid, 4-O-α-D-glucuronosyl-

D-galactose whilst A. nilotica yields two more aldobiouronic acids namely: 6-O-β-(4-O-methyl-D-glucuronosyl)-D-galactose and 4-O-α-(4-O-methyl-D-glucuronosyl)-D-galactose. The last two aldobiouronic acids have been isolated from gums containing relatively high methoxyl content e.g. mesquite gum (86) and gum myrrh (87). Whether the existence of aldobiouronic acids other than 6-O-β-D-glucuronosyl-D-galactose in A. karoo and A. nilotica implies a fundamental difference in the structure of the gum from that of other Acacia species is not yet decided.

A. SENEGAL:

The gum from A. senegal (syn. Verek) (gum arabic) has probably been subjected to more extensive investigation than any other gum in the Acacia genus (14, 69-71). In structure it is fairly complex. The principal sugar residues present in the molecule are D-galactose L-arabinose, L-rhamnose and D-glucuronic acid. As far as is known at present, the galactose units of the main chain are glycosidically linked through the 1 and 3 positions, and the branches are attached through carbon atom 6. The rhamnose is present entirely as end-groups, and the arabinose partly as arabofuranose end groups, and partly linked through the 1 and 3 positions. Partial hydrolysis of gum arabic gives some 3-O-β-L-arabopyranosyl-L-arabinose (88) and the aldobiouronic acid isolated is 6-O-β-D-glucuronosyl-D-galactose. No unique structure can be postulated for the gum, but the following is one of the many possibilities (89):



Gal = D-galactopyranose

Ara = L- Arabinose (mostly in furanose form)

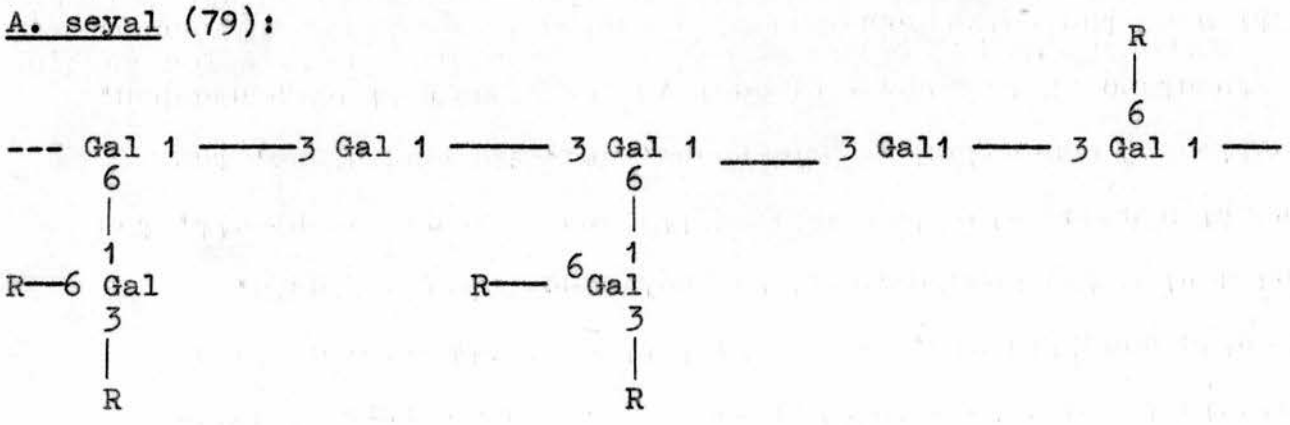
R = L-rhamnose (pyranose form)

G.A.= D-glucuronic acid

A. PYCNANTHA :(72, 73)

A. Pycnantha is another member of the genus which has been subject of detailed methylation studies; it seems to be rather more simple than gum arabic in structure. It is less highly branched, some rhamnose units are attached directly to the backbone of the molecule, and the glucuronic acid occurs exclusively as end group. Like A. senegal, the gum from A. pycnantha contains a backbone of 1:3-linked β-D-galactose residues to which is attached a complex system of side chains, resembling, but clearly not identical in structure to, those present in gum arabic (85).

A. seyal Del (79) shows similar structural features to those of A. senegal and A. pycnantha and in many ways seems to be intermediate between them. Again the main chain is composed of 1:3-linked D-galactose residues with side chains of 1:6 linked D-galactose residues and such side chains are attached to approximately every second main chain galactose residue. As in A. pycnantha the acid disaccharides grouping occurs exclusively as end groups. The acid-labile groups appear to be attached to both C atoms 3 and 6 of the galactose units. The following is a general representation of the main structural features of A. seyal (79):



R = Araf 1---- or Rhp 1 --- or Galp 1 ——3 Araf ---

or Arap 1 —— 3 Araf ---- or G. A. 1 ----

Comparison of the properties and composition of the Acacia gums so far investigated and results from methylation studies are shown in Tables I - IV.

Table I

Properties of Acacia gums

Species	Equivalent Weight	$[\alpha]_D$ in water	Ref.
A. senegal	1000 - 1400	-28°	(69)
A. seyal del	1300	+60°	(79)
A. nilotica	1900	+108°	(P52)
A. karoo	1660	+54°	(75)
A. mollisima	1880	-49°	(76)
A. pycnantha	3700	-8°	(72)
A. cyanophylla	740	-20°	(74)

Table II

Composition of Acacia gums.

Molar proportions of sugar residues.

Species	Gal	Ara	Rha	G.A.	Ref.
A. senegal	3	3	1	1	(69,70)
A. seyal del	10	12	1	3	(79)
A. nilotica	57	72	-	11	(P45)
A. karoo	28	24	1	6	(75)
A. mollissima	5	6	1	1	(76)
A. pycnantha	40	20	1	6	(72)
A. catechu	9	4	3	3	(78, 91)
A. cyanophylla	11	2	5	5	(74)
A. sundra	41	27	14	18	(77, 92)

Key: Gal = D-galactose Rha = L-rhamnose
Ara = L-arabinose P = pyranose
f = furanose G.A. = D-glucuronic acid
4-O-me G.A. = 4-O-methyl-D-glucuronic acid

Table III

Oligosaccharides isolated from the partial hydrolysis of *Acacia* gums

species	Arap1-3Ara	Gal1-3Ara	Gal1-3Gal	Gal1-6Gal	G.A.1-6Gal
senegal	+	+	+	+	+
cyanophylla		+			+
mollissima	+		+		+
karoo	+	+			+
pycnantha			*		*
seyal					+
sundra					+
catechu					+
nilotica	+		+	+(?)	+

Species	G.A.1-4Gal	4-O-meG.A.1-6Gal	4-O-me G.A.1-4Gal	Ref.
senegal				69,71
cyanophylla				74
mollissima		+		76
karoo	+			75
pycnantha				72,73
seyal				79
sundra				77
catechu				82
nilotica	+	+	+	Part III

Table IV

Products isolated after hydrolysis of methylated Acacia gums

Species	Gluc.A.	Gal.	Ara	Rha	Ref.
senegal	2,3- 2,3,4-	2,4- 2,3,4,6-	2,5- 2,3,5- 2,3,4-	2,3,4-	69
seyal	2,3,4-	2- 2,4- 2,4,6- 2,3,4,6- 2,3,4- (trace)	2,3- 2,5- 2,3,5- 2,3,4-	2,3,4-	79
pycnantha	hexa-methyl ether of G.A.1-6Gal	2-, 2,3- 2,5-,2,4- 2,4,6- 2,3,4- 2,3,4,6-	2,3- 2,5- 3,5- (trace) 2,3,5-	2,3,4-	90
catechu (degraded gum)	2,3,4-	4,6- 2,3,4- 2,3,4,6-			91
sundra (degraded gum)	2,3,4-	2,4- 2,3,4- 2,4,6-			92
nilotica	2,3,4-	2,4- 2,3,4- 2,4,6- 2,3,4,6-	2,3- (?) 2,5- 2,3,4- 2,3,5-		Part III

OBJECT OF THE PRESENT INVESTIGATIONS:

In this work it was intended to carry out a comparative analytical study of:

(a) 16 different specimens of Acacia senegal gum with a view to ascertaining any variation due to (i) soil (ii) season, and (iii) the state and shape of the gum nodules.

(b) 7 different, previously unstudied, Acacia species, and

(c) 10 different specimens (nodules) of Acacia nilotica.

The latter part of this work consists of a detailed structural investigation of a typical representative nodule of Acacia nilotica, which in comparison with previously studied Acacia gums, shows a relatively very low nitrogen content, a high positive ~~optical~~ specific rotation, and a relatively high methoxyl content. These differences indicated that this species of gum might differ structurally from the species of Acacia gum previously studied.

EXPERIMENTAL

General Methods:

Moisture content was found by heating to constant weight at 105°C.

Ash content was found by heating in a muffle at 550°C; constant weight was normally reached in 5 hours.

Nitrogen content was found by a semi-micro Kjeldhal method.

Uronic acid and methoxyl contents were determined by vapour-phase infra-red method (93).

Viscosity measurements were made using a suspended level dilution viscometer in a thermostat at 25.0 ± 0.01°C.

Free titrable acidity was found by direct titration with 0.02N sodium hydroxide to phenolphthalein end-point in stream of carbon dioxide-free nitrogen.

Reducing power was determined by the Somogyi method (94). The total free reducing sugars were calculated as L-arabinose.

Optical rotations of substances were measured in aqueous solution (unless otherwise stated) at 20 ± 1°C.

Paper chromatography: descending chromatography used for qualitative and quantitative work, was carried out on Whatman No.1 filter paper, unless otherwise stated. The following solvent systems (v/v) were employed:

(A) Butan-1-ol-ethanol-water (4:1:5, upper layer).

(B) Butan-1-ol-pyridine-water-benzene (5:3:3:1, upper layer).

(C) Ethyl acetate-pyridine-water (10:4:3)

(D) Ethyl acetate-acetic acid-formic acid-water (18:3:1:4)

(E) Ethyl acetate-acetic acid-water (9:2:2)

(F) Butan-1-ol-acetic acid-water (4:1:5, upper layer)

(G) t-Amyl alcohol : n-propanol : water (4:1:1.5)

Spray Reagents:

(i) Aniline oxalate: chromatograms of reducing sugars were sprayed with a saturated aqueous solution of aniline oxalate, and developed by heating for about 5 minutes at 150°C, when various characteristic colours appeared.

(ii) Silver nitrate: reducing sugars and sugar alcohol chromatograms were developed by immersion in saturated solutions of silver nitrate in acetone and spraying with 1·0N sodium hydroxide in ethanol. The black spots indicating the position of of sugars appeared in about two minutes. The papers were then treated with aqueous sodium thiosulphate solution and washed in running water.

All sugars (whenever possible) were identified by comparison with reference sugars run on the same chromatograms.

The rate of movement of methylated sugars was given relative to that of 2,3,4,6-tetra-O-methyl-D-glucose on No. 1 paper in solvent(A), and referred to as the R_G value. That of other sugars was given relative either to the solvent front (R_F), or to some convenient standard sugar (e.g. R_{Gal} = relative to D-galactose).

Separation of Sugars on Thick Paper:

Whatman 3MM was used for preparative separation of sugars. The positions of the sugars after separation was ascertained by cutting out and spraying a narrow centre strip. The appropriate parts of the filter sheet were then cut out and eluted with cold water until the eluate gave a blank reading with phenol-sulphuric acid reagent (28).

Evaporations were carried out under reduced pressure at or below 40°C.

Small-scale Hydrolyses:

These were carried out by heating the sample with the given normality of the acid in a sealed tube on a boiling water-bath. Where sulphuric acid was used, barium hydroxide solution was added until the solution was almost neutralised; this was completed by addition of solid barium carbonate. The solution was filtered and the filtrate evaporated to dryness. If uronic acids were present the filtrate was first stirred with cation exchange resin (Amberlite IR-120,(H)) for ca. ½ hour to convert the barium salt of the acid into the free acid.

Hydrochloric acid hydrolysates were neutralised with silver carbonate and filtered. The residue was washed thoroughly with water, filtered and the filtrates combined and evaporated to dryness. Methanolysis was carried out by heating the sugars with dry methanolic hydrogen chloride (2 - 6%) in a sealed tube for 6 -12 hours on a boiling water bath. The solution was neutralised with silver carbonate and filtered, the silver carbonate and silver chloride were washed with acetone, and the combined filtrates taken to dryness under vacuum.

Demethylations (95) were carried out by dissolving the methylated sugar (5 mg) in dry dichloromethane (2 ml) cooled in acetone / CO_2 mixture. Borontrichloride (2 ml), previously cooled to -80°C, was then added. The mixture was then sealed in a glass tube and kept at -80° for 30 minutes before it was allowed to come to room temperature. After a further 16 hours, the tube was opened and the remaining liquid mixture was removed

under reduced pressure. The remaining solid was extracted with methanol (3 x 3 ml) then examined by paper chromatography.

Column Chromatography:

(a) Cellulose columns were prepared from a slurry of cellulose in acetone. The column was then washed with ethanol, water and finally with the solvent to be used. Butan-1-ol for use on column was purified by refluxing for 2 hours with potassium hydroxide (1% w/v), and distilling; light petroleum (b.p. 100 - 120°C) was shaken several times with concentrated sulphuric acid (10% w/v), washed free of acid with distilled water and distilled.

Fractionation was achieved by collecting the eluate in tubes on an automatic turn-table. At suitable intervals the contents of a tube were evaporated to dryness and examined by paper chromatography. Fractions were bulked accordingly, and evaporated to dryness, and the syrups obtained were cleaned by dissolving in water and treating with charcoal. The final filtrate was taken to dryness, dried over phosphorous pentoxide, and weighed.

(b) Charcoal-Celite column: This was prepared as follows: Activated charcoal was washed by decantation with hot water. Celite (grade 545) was washed with hot concentrated hydrochloric acid:water (1:1) and then successively with water, 3% aqueous sodium bicarbonate, and water. Equal weights of charcoal and celite were mixed well in a slurry which was then poured into a glass column. Before applying the sugar mixture, the column was thoroughly washed with water.

(c) Diethylaminoethylcellulose(DEAE) Column (96): The DEAE-cellulose powder (80 g.) was first washed with 0.5N hydrochloric acid (3 x 500 ml) followed by 0.5N sodium

hydroxide (3 x 500 ml). The DEAE-cellulose was stirred with the acid (or base) for 10-15 minutes, allowed to settle and the turbid solution decanted off. The DEAE-cellulose was then washed with distilled water until the washing was neutral. A perforated disc was placed at the bottom of the column, then a layer of glass wool followed by a layer of silver sand. The column was then nearly filled with distilled water. The DEAE-cellulose was transferred into the column by means of a syphon, the DEAE-cellulose being continually stirred in a large beaker. A layer of glass wool was placed at the top as well. The initial generation and subsequent regeneration of the DEAE-cellulose in the phosphate form was done by eluting the column with 2L of 0.5M sodium dihydrogen phosphate solution (pH 6, adjusted by adding 1N NaOH) followed by equilibration with 1l of 0.05M solution of the same buffer at the same pH. The rate of flow of the column during this operation was adjusted to ca. 40 ml/hr.

The polysaccharide (300 - 500 mg) was dissolved in water (10 ml) run onto the column and left to stand over-night, so that the polysaccharide got fully soaked into the ion-exchange cellulose. The column was first eluted successively with (a) 0.025M (500 ml), (b) 0.05M (500 ml), (c) 0.10M (500 ml), (d).25M (500 ml), (e) 0.50M (500 ml) of NaH_2PO_4 buffer at pH 6. Any remaining polysaccharide was finally eluted with 500 ml each of (a) 0.05M, (b) 0.10M, (c) 0.25M, (d) 0.50M, NaOH solution.

Fractions (40 ml) were screened by the phenol-sulphuric acid method (3), using a calibration curve based on the polysaccharide itself. Each fraction was dialysed in running water (2 days) further de-ionised on Amberlite IR-45 (OH) and IR-120 (H), and finally taken down to a small volume and freeze-dried.

Phenol-Sulphuric Acid Method for Estimation of Mono- and polysaccharides (3):

The reagent was made by dissolving 5 g. of Analar phenol in 100 ml of copper-free distilled water. Various volumes (0.1 - - 2.0 ml) of the mono- or polysaccharide solution were pipetted into test tubes (15 x 2 cm) and diluted with 2 ml of distilled copper-free water. 1 ml of the phenol solution was added to each, followed by 5 ml of Analar concentrated H_2SO_4. The tubes were allowed to stand for 10 minutes, shaken and placed for 10 - 20 minutes in a water bath at 30°C. The optical density was then measured with a Unicam SP 500 at 485 mμ for polysaccharides against a blank prepared with 2 ml of water. The standard curve was obtained by plotting a known weight of polysaccharide (mg/ml) against the optical density. Similar standard curves for L-arabinose, D-galactose, L-rhamnose, D-ribose and D-glucuronic acid were obtained.

Chromotropic Acid Reagent for Formaldehyde Estimation (97):

1 g. of purified sodium salt of chromotropic acid was dissolved in hot water (100 ml). After adding 0.1 g of $SnCl_2$ the solution was diluted to 500 ml with H_2SO_4 (66% v/v), and the solution stored in the dark in a brown bottle. All operations were carried out in the dark. For use, 9 ml of the reagent was added to 1 ml of reaction mixture, then heated for ½ hour at 100°C and then cooled. 2 ml of 4.6% thiourea was added and the optical density read on a Unicam SP 1300 at 570 mμ. A calibration curve was obtained for formaldehyde using D-glucose and ½% sodium meta-periodate solution at pH 8.

Phenylosazones: The sugar (ca. 10 mg) was heated for 30 minutes on a boiling water bath with 0.01 ml of each of phenyl-hydrazine and glacial acetic acid, water (0.25 ml) and a drop of

saturated sodium bisulphite solution. On cooling and adding water (ca. 1.5 ml) the phenylosazone was precipitated and was recrystallised from boiling water.

Aldonolactones: The methylated sugar (10 - 50 mg) was dissolved in water (2 ml), bromine (5 - 20 drops) was added, and the mixture was kept in the dark at room temperature for two days. Excess bromine was removed by aeration and the solution evaporated to dryness. The residue was extracted with ether and the lactone recrystallised by slow evaporation of an ethanolic solution.

Aldonamides: The lactone, prepared as above, was dried in a vacuum dessicator, dissolved in dry methanolic ammonia (prepared by bubbling ammonia gas into dry, ice-cooled methanol, until saturation) and left in the ice-box for 2 days. Evaporation of the solvent gave the crystalline amide which was recrystallised from the stated solvent.

Aniline derivatives: The methylated sugar was refluxed with an equimolecular amount of freshly distilled aniline in dry ethanol for 30 minutes in the dark. Evaporation of the solvent gave the aniline derivative, which was generally recrystallised from ethyl acetate.

Reduction with potassium borohydride: The substance (20 mg) was dissolved in water (5 ml) and potassium borohydride (100 mg) in water (5 ml) was added dropewise. The reaction mixture was left at room temperature overhight. De-ionisation was effected by treatment with Amberlite resin IR-120 (H), followed by several evaporations with methanol.

PART II

COMPARATIVE ANALYTICAL STUDIES ON THE GUM EXUDATES FROM SOME ACACIA SPECIES

COMPARATIVE ANALYTICAL STUDIES ON THE EXUDATE FROM SOME ACACIA SPECIES

(A) Comparative Examination of Sixteen A. senegal Samples:

Origin and description of samples: Sixteen representative samples of A. senegal gum of authenticated origin have been examined as crude gum, and also after purification by electrodialysis. The examination was carried out to see if there was any variation in the composition or properties of the gum (i) because of growth on different types of soil, (ii) because of seasonal variation, including variation in different pickings (crops) within any one season. The investigation was also extended to test if any variation occured between nodular and non-nodular forms of A. senegal gum.

The samples, taken only from trees which could be authenticated, were collected and despatched individually by the Gum Research Officer, Department of Forests, Republic of the Sudan. Samples Im- V were nodular specimens collected at Quala en Nahal (heavy clay soil), Kassala Province in Eastern Sudan; samples VI and VII from Umm Ruaba (sandy soil, Kordofan Province), and samples VIII - XII from Goz el Ganzara (sandy soil) also in Kordofan, Western Sudan. Samples XIII, XIV and XV were specimens of non-nodular form. Sample XVI was a specimen of "hennawi" i.e. dark brown nodules obtained as natural exudate from the lower stem near the roots of trees.

(i) Samples from Quala en Nahal (heavy clay soil):

I	SS 12	first picking	1960
II	SS 13	second picking	1960
III	SS 14	third picking, 1st April,	1960
IV	SS 15	fourth picking, 19th April,	1960

V SS40 early picking 1962

(ii) Samples from Umm Ruaba (sandy soil):

VI SS16 early picking 1960

VII SS17 late picking 1960

(iii) samples from Goz el Ganzara (sandy soil):

VIII SS18 representative sample 1960

IX SS41A first picking, 19th January, 1962

X SS41B second picking,4th February,1962

XI SS41C third picking, 19th February, 1962

XII SS41D fourth picking 5th March, 1962

(iv) Non-nodular forms:

XIII SS26(a): flat ribbon, opaque appearance

XIV SS26(b): narrow filamenteus form of opaque appearance

XV SS26(c): filamentous form of bright, strongly refractive appearance.

XVI SS65 : "hennawi" usually dark brown, brittle, nodules.

All samples were hand-cleaned, freed from contaminating pieces of bark, sandy pieces etc. To ensure representative sampling, the specimen as received was either dissolved, filtered and the solution freeze-dried, or, alternatively, finely ground. Difficulty in grinding is usually overcome by heating gently (80 - 85°C) to effect dehydration, but no heating was necessary with any of the specimens studied.

Having obtained a finely ground sample, the following analyses were carried out on the crude and on the purified, i.e. electrodialysed, gum:-

(1) Determination of moisture content (to permit correction to dry weight basis).

(2) Determination of ash content; samples of ash were retained for possible later analysis for inorganic elements present (Table IX).

(3) Determinationof % nitrogen.

(4) Determination of limiting viscosity number, and analyses on 3% aqueous solutions for:-

(a) % insoluble in cold water.

(b) pH

(c) free titrable acidity,

(d) reducing power,

(e) optical rotation, and

(5) Determination of % uronic acid and % methoxyl.

The analytical methods employed have already been described, and the results are shown in Tables V - IX.

Table - V

Determinations on crude samples of A. senegal.

Sample	I	II	III	IV	V	VI	VII	VIII
% moisture	12.9 12.8	13.5 13.4	13.3 13.3	13.1 13.2	12.7 12.7	13.3 13.2	13.7 13.4	12.5 12.3
% ash	3.64 3.65	3.35 3.39	3.07 3.08	2.90 2.92	3.60 3.64	3.05 3.06	3.50 3.55	3.69 3.68
% nitrogen	0.33. 0.34	0.34 0.34	0.36 0.36	0.36 0.35	0.34 0.34	0.39 0.40	0.39 0.40	o.39 0.39
% uronic anhydride	16.0	16.0	16.2	16.0	16.2	16.0	16.0	16.0
% methoxyl	0.37	0.31	0.30	0.35	0.30	0.32	0.35	0.30

Sample	IX	X	XI	XII	XIII	XIV	XV	XVI
% moisture	12.4 12.5	13.0 12.9	13.0 13.0	13.2 13.3	13.4 13.4	13.3 13.1	13.1 13.1	13.6 13.6
% ash	4.16 4.17	3.98 3.98	3.93 3.93	3.81 3.81	3.88 3.89	3.68 3.72	3.95 3.98	5.66 5.68
% nitrogen	0.42 0.42	0.41 0.42	0.39 0.38	0.38 0.38	0.40 0.40	0.34 0.34	0.39 0.39	0.27 0.27
% uronic anhydride	16.2	16.2	16.2	15.8	15.8	16.0	16.2	14.0
% methoxyl	0.31	0.32	0.32	0.30	0.20	0.20	0.20	0.19

Table - VI

Determinations on 3% aqueous solutions of A. senegal crude gum samples

Sample	I	II	III	IV	V	VI	VII	VIII
% cold water insoluble	0.35	0.32	0.44	0.17	0.60	0.35	1.0	1.5
pH	4.49	4.50	4.52	4.58	4.52	4.53	4.61	4.66
Free titrable acidity (ml of 0.021N NaOH per 10 ml)	1.00 1.00	0.90 0.90	0.90 0.89	0.79 0.79	0.96 0.96	0.89 0.91	0.80 0.81	0.90 0.86
% free reducing sugar (as arabinose)	0.27 0.27	0.30 0.30	0.30 0.30	0.32 0.32	0.42 0.41	0.33 0.33	0.43 0.43	0.25 0.24
Flow-time at 25°C(secs.) (water= 212 secs.) Hence $\frac{t - t_o}{t_o}$	575 576 1.81	510 512 1.49	483 483 1.36	455 455 1.15	603 603 1.85	430 430 1.10	426 426 1.08	559 559 1.73

Sample	IX	X	XI	XII	XIII	XIV	XV	XVI
% cold water insoluble	1.6	1.3	0.49	0.17	0.43	0.23	0.30	0.51
pH	4.65	4.63	4.63	4.65	4.51	4.48	4.40	6.25
Free titrable acidity (ml of 0.021N NaOH per ml)	0.90 0.86	0.89 0.93	0.92 0.94	0.96 0.97	0.76 0.76	0.73 0.72	1.00 1.02	0.23 0.25
% free reducing sugar (as arabinose)	0.29 0.28	0.31 0.31	0.25 0.26	0.31 0.31	0.28 0.27	0.22 0.24	0.16 0.17	0.07 0.08
Flow-time at 25.0°C (water=212 secs.)	613 613	633 633	713 713	658 657	740 740	602 603	659 658	455 456
Hence $\frac{t - t_o}{t_o}$	1.99	2.09	2.38	2.10	2.49	1.85	2.11	1.15

Table - VII

Determinations on purified material.

Sample	I	II	III	IV	V	VI	VII	VIII
% nitrogen	0.31 0.31	0.31 0.31	0.31 0.31	0.31 0.31	0.31 0.31	0.32 0.32	0.32 0.33	0.32 0.33
% uronic anhydride	14.5	14.5	14.4	14.4	14.6	14.6	14.6	14.6
% methoxyl	0.34	0.33	0.34	0.35	0.36	0.36	0.35	0.34

Sample	IX	X	XI	XII	XIII	XIV	XV	XVI
% nitrogen	0.36 0.36	0.36 0.36	0.32 0.32	0.31 0.32	0.33 0.32	0.33 0.32	0.32 0.31	0.52 0.52
% uronic anhydride	14.6	14.6	14.6	14.6	14.3	14.7	14.5	13.3
% methoxyl	0.34	0.35	0.35	0.35	0.30	0.40	0.30	0.26

Table - VIII

Determinations on 3% aqueous solutions of purified material.

Sample	I	II	III	IV	V	VI	VII	VIII
pH	2.56	2.56	2.56	2.57	2.52	2.50	2.53	2.50
% free reducing sugars	0.31 0.31	0.24 0.24	0.43 0.43	0.36 0.36	0.48 0.48	0.24 0.24	0.34 0.35	0.20 0.21
$[\alpha]_D^{20^\circ}$	-28°	-27°	-29°	-28°	-27°	-27°	-27°	-27°
Flow-time at 25.0°C (Water=186 sec.) in 4% NaCl solution.	326 327	290 291	313 313	294 295	354 354	303 303	310 310	390 390
Hence $\frac{t - t_o}{t_o}$	0.76	0.56	0.68	0.58	0.90	0.63	0.67	1.10
Free titrable acidity (ml of 1N NaOH per gm.)	0.799 0.805	0.683 0.680	0.692 0.693	0.690 0.693	0.708 0.704	0.689 0.689	0.648 0.651	0.717 0.718
Hence App. equiv. wt.	1252 1242	1464 1471	1445 1443	1449 1443	1412 1420	1451 1451	1543 1536	1395 1393
Hence %U.A.A.	14.1	12.0	12.2	12.2	12.4	12.1	11.4	12.6

App. equiv. wt. = Apparent equivalent weight

U.A.A. = Uronic acid anhydride

Table continued on p 39

Table VIII continued:

Sample	IX	X	XI	XII	XIII	XIV	XV	XVI
pH	2.47	2.50	2.50	2.53	2.51	2.49	2.53	2.60
% Free reducing sugars	0.27 0.28	0.27 0.27	0.36 0.36	0.28 0.28	0.27 0.27	0.23 0.22	0.16 0.16	0.08 0.07
$[\alpha]_D^{20^\circ}$	-28°	-28°	-28°	-27°	-27°	-30°	-27°	-27°
Flow-time at 25.0°C (water = 186 secs.) in 4% NaCl solution	424 425	384 385	401 400	362 363	385 385	386 386	376 376	376 375
Hence $\frac{t - t_o}{t_o}$	1.28	1.07	1.18	0.94	1.07	1.07	1.02	1.02
Free titrable acidity (ml 1N NaOH per gm.)	0.832 0.832	0.808 0.805	0.805 0.798	0.776 0.777	0.764 0.757	0.794 0.789	0.694 0.691	0.706 0.706
Hence App. equiv. wt.	1202 1202	1238 1242	1242 1253	1289 1287	1309 1321	1259 1267	1441 1447	1416 1416
Hence % U.A.A.	14.6	14.2	14.1	13.7	13.5	14.0	12.2	12.4

Table - IX

Ash analysis of 12 A. senegal samples (98).

Gum sample	X-ray fluorescence		Flame photometry		EDTA
	% Fe	% Ca	% K	% Na	% Mg
I	0.03	5.7	13.6	0.45	4.0
II	0.04	5.3	15.5	0.36	4.3
III	0.05	6.1	13.4	0.37	5.0
IV	0.05	6.8	13.4	0.37	6.8
V	0.03	7.2	15.0	0.41	6.6
VI	0.03	7.9	16.6	0.45	5.1
VII	0.03	8.5	17.4	0.29	4.7
VIII	0.04	5.0	16.6	0.46	4.9
IX	0.03	9.3	16.5	0.41	4.2
X	0.03	7.5	16.8	0.45	4.2
XI	0.04	7.6	17.4	0.37	4.4
XII	0.02	7.6	11.5	0.49	4.9

(B) Comparative Analytical Studies of Gum Samples from Seven Different Acacia Species.

Fig. I and tables (X - XVII) present the results of analytical studies carried out on the gum exudates from the following species of Acacia which had not been investigated previously:

(a) A. nilotica (SS 62a) *

(b) A. arabica

(c) A. campylacantha

(d) A. drepanolobium

(e) A. dealbata

(f) A. seyal var fistula (SS 60e)

(g) (i) A. nubica (SS 49), (ii) A. nubica (SS50) and (iii) A. nubica (SS 55).

All the samples were obtained from the Sudan. The analytical methods employed have already been described in Part - I of this thesis. Results are given both for the crude and after purification by electrodialysis.

* SS = Sudanese sample

Table - X

Composition of seven *Acacia* gum exudates.

Gum	Acid	D-galactose	L-arabinose	Me-pentose
A. arabica	4-MeGlu;Glu	+	+	Rh (trace)
A. campylacantha	Glu	+	+	Rh
A. drepanolobium	Glu	+	+	Rh
A. dealbata	Glu	+	+	Rh
A. seyal var fistula	4-MeGlu;Glu	+	+	Rh (trace)
A. nubica	Glu	+	+	Rh (trace)
A. nilotica	4-MeGlu;Glu	+	+	Rh (trace)

Glu = D-glucuronic; Rh = L-rhamnose

Table - XI

Percent composition of some Acacia gums.

Gum	Glu acid %	Gal %	Ara %	Rh % (99)
A. arabica	7.6	36	54	0.4
A. campylacantha	8.2	52	26	7.0
A. drepanolobium	9.0	28	60	1.0
A. dealbata	7.7	41	41	6.0
A. fistula	9.3	36	49	trace
A. nubica	6.4	33	56	0.6
A. senegal (Henn)	14.0	50	28	5.0
A. nilotica	9.2	44	46	0.4

Table - XII

Absolute weights of sugars in some Acacia samples.

Gum	Wt. of gum taken (mg.)	Wt. of Ribose added (mg.)	Wt. of Ribose recovered. (mg.)	Wt. of Glu. acid (mg.)	Wt. of Gal. (mg.)	Wt. of Ara. (mg.)	Wt. of Rh. (mg.)
A. arabica	74.0	13.2	11.1	7.4	26.6	40.0	-
A. campylacantha	74.3	13.5	12.0	9.2	37.0	18.5	6.1
A. drepanolobium	51.7	13.5	12.1	5.8	14.4	31.0	-
A. dealbata	63.3	16.4	15.1	7.4	26.0	26.0	3.7
A. fistula	43.6	8.4	8.0	5.5	16.1	21.5	-
A. nubica	54.7	13.3	12.8	4.8	18.1	31.2	-
A. senegal (Henn)	46.7	10.4	9.6	7.8	23.5	13.0	2.6

Table - XIII

Molar ratios of anhydro sugars in some Acacia gums.

Gum	D-Glu.acid	D-Gal.	L-Ara.	L-Rh.
A. arabica	1	4	6	-
A. campylacantha	1	4	2	1
A. drepanolobium	2	5	11	-
A. dealbata	2	7	7	1
A. fistula	1	3	5	-
A. nubica	2	7	13	-
A. senegal (Henn)	3	9	5	1
A. nilotica	11	57	72	-

Table - XIV

Determinations on crude samples.

Gum	nil.(SS62A)	ara.	camp.	drep.	deal.
Moisture (%)	10.3 10.3	11.8 11.9	13.4 13.4	13.2 13.2	11.4 11.6
Ash (%)	2.46 2.50	2.38 2.38	2.91 2.94	2.50 2.53	2.94 2.95
Nitrogen(%)	0.08 0.08	0.08 0.08	0.35 0.34	1.24 1.26	0.66 0.65
Uronic anhydride (%)	9.6	12.0	12.4	9.0	7.7
Methoxyl (%)	1.05	0.87	0.2	0.4	0.26

Gum	fist.	nub. (SS49)	nub. (SS50)	nub. (SS55)
Moisture (%)	9.42 9.50	10.4 10.5	11.1 -	9.2 -
Ash (%)	2.90 2.92	1.51 1.57	1.51 1.52	1.54 1.54
Nitrogen (%)	0.07 0.07	0.20 0.19	0.23 0.22	0.21 0.21
Uronic anhydride (%)	9.3	6.4	7.5	7.6
Methoxyl (%)	0.90	0.10	0.10	0.11

Table - XV

Determinations on 3% aqueous solutions of crude material.

Gum	nil. (SS 62a)	arab.	camp.	drep.=	deal.
Cold water insoluble	0.25	5.2	0.69	18.6	6.9
pH	4.70	4.68	4.95	4.86	4.32
Free reducing sugars (%)	0.16 0.16	0.21 0.21	0.11 0.12	0.36 0.38	0.86 0.86
Free titrable acidity (ml. 0.02N NaOH per 10 ml.)	0.50 0.50	0.51 0.51	0.30 0.30	0.90 0.91	1.47 1.48
Flow-time at 25.0°C (water= 212 sec.)	360 361	421 421	390 388	427 426	765 765
$\therefore \frac{t - t_o}{t_o}$	0.70	0.99	0.84	1.01	2.61
$[\alpha]_D^{20^\circ}$	+106°	+97°	-3°	+74°	-24°

Gum	fist.	nub. (SS49)	nub. (SS50)	nub. (SS55)
Cold water insoluble	0.12	1.4	1.5	1.9
pH	4.74	4.70	4.74	4.84
Free reducing sugars (%)	0.41 0.41	0.12 0.12	0.25 0.24	0.16 0.16
Free titrable acidity (ml. 0.02N NaOH per 10 ml.)	0.79 0.80	0.49 0.50	0.64 0.64	0.60 0.60
Flow-time at 25.0°C (water= 212 secs.)	521 520	348 348	346 348	354 354
$t - t_o / t_o$	1.45	0.64	0.64	0.67
$[\alpha]_D^{20^\circ}$	+60°	+98°	+100°	+99°

Table - XVI

Determinations on purified material.

Gum	nil. (SS63a)	nil. (62 a)	arab.	camp.	drep.
Moisture (%)	2.86	0.01	1.81	3.00	6.30
Ash (%)	0.04	0.015	0.03	0.047	0.00
Nitrogen (%)	0.02 0.02	0.021 0.020	0.064 0.068	0.27 0.28	1.10 1.13
Uronic anhydride (%)	10.9	9.2	7.8	8,2	9.0
Methoxyl (%)	1.44	0.96	0.88	0.40	0.40

Gum	deal.	fist.	nub. (SS49)	nub. (SS50)	nub. (SS55)
moisture (%)	6.2	2.74	4.1	4.1	4.2
Ash (%)	0.026	0.00	0.026	0.02	0.01
Nitrogen (%)	0.60 0.61	0.059 0.051	0.21 0.21	0.21 0.21	0.16 0.15
Uronic anhydride (%)	7.8	9.3	6.4	6.1	6.4
Methoxyl (%)	0.35	0.90	0.20	0.12	0.14

Table - XVII

Determinations on 3% aqueous solutions of purified material.

Gum	nil.(?) (63a)	nil. (62a)	arab	camp.	drep.
pH	2.61	2.87	2.89	2.60	2.88
Free reducing sugars (%)	0.16 0.16	0.05 0.05	0.13 0.13	0.11 0.12	0.36 0.38
Free titrable acidity (ml. 0.022N NaOH per5ml.)	4.08 4.08	3.58 3.62	2.86 2.87	3.37 3.36	3.32 3.30
Ml. of N NaOH per 1 gm	0.600 0.600	0.528	0.421 0.422	0.495 0.494	0.488 0.485
Hence Apparent equivalent weight	1667 1667	1894	2375 2370	2020 2024	2049 2062
Hence apparent U.A.A. (%)	10.6 10.6	9.3	7.41 7.43	8.71 8.70	8,60 8.54
Flow-time at 25.0°C in 4% NaCl solution (water =186 sec.)	303 304	285 285	382 382	328 328	417 418
Limiting flow time number $\frac{t - t_o}{Ct_o}$ at 25.0°	10.4	n.d.	12.5	16.0	16.6
$[\alpha]_D^{20^\circ}$	+54°	+108°	+100°	-3°	+75°

n.d. = not determined

Table continued on p50

Table XVII (continued)

Gum	deal.	fist.	nub. (SS49)	nub. (SS50)	nub. (SS55)
pH	2.64	2.69	2.95	3.00	2.90
Free reducing sugars (%)	0.86 0.86	0.30 0.31	0.36 0.37	0.10 0.12	0.15 0.13
Free titrable acidity (ml. 0.022N NaOH per 5 ml.)	3.70 3.69	4.44 4.44	2.26 2.24	1.92 1.89	2.60 2.60
Ml. of N NaOH per 1 gm.	0.544 0.542	0.653 0.653	0.331 0.329	0.282 0.277	0.381 0.381
Hence apparent equivalent weight	1838 1845	1531 1531	3021 3040	3546 3610	2625 2625
Hence apparent U.A.A. (%)	9.57 9.54	11.5 11.5	5.83 5.79	5.00 4.90	6.70 6.70
Flow-time at 25.0°C in 4% NaCl solution (water= 186 secs.)	534 534	402 401	293 294	294 295	282 280
Limiting flow time number, $\frac{t - t_o}{Ct_o}$ at 25.0°	21.5	19.4	9.8	n.d.	n.d.
$[\alpha]_D^{20^\circ}$	-25°	+61°	+100°	+100°	+100°

(C) Comparative Analytical Studies on Ten A. nilotica gum Samples

Samples 62 (a), (b), (c) and (d) are nodules from separate individual small trees of A. nilotica. Samples 63 (a), (b), (c), (d), (e) and (f) are nodules from separate individual large trees of A. nilotica. The samples, which were natural exudates, were picked on 3rd February, 1963 in Hawata, Kassala Province in the Eastern Sudan. The colour of the fragmented nodules ranged from colourless to deep brown. All samples were purified by electrodialysis. Results of the analysis are shown on Tables (XVIII - XXII).

Table - XVIII

Determinations on purified A. nilotica samples.

	S.S. 62				S.S. 63					
	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)	(e)	(f)
Moisture (%)	0.01	0.02	0.05	0.03	5.1	3.0	0.31	0.05	0.01	0.15
Nitrogen (%)	0.02	0.02	0.02	0.03	0.02	0.01	0.03	0.01	0.01	0.01
	0.02	0.02	0.02	0.03	0.02	0.01	0.03	0.01	0.01	0.01
U.A.A. (%)	9.2	9.2	9.4	9.3	10.9	9.3	9.2	9.3	9.1	9.3
Methoxyl (%)	0.96	0.75	1.25	1.18	1.44	1.30	1.25	1.03	1.13	1.28

Table - XIX

Determinations on 3% aqueous solutions of purified *A. nilotica* samples.

	S.S. 62			
	(a)	(b)	(c)	(d)
pH	2.87	2.87	2.87	2.86
Free reducing sugars (%)	0.10	0.05	0.13	0.05
Free titrable acidity (ml. 0.022N NaOH per 5 ml.)	3.58 3.62	3.58 3.61	3.65 3.66	3.53 3.52
Ml. of 1N NaOH/gm.	0.528	0.528	0.537	0.519
Hence apparent equivalent weight	1894	1894	1862	1927
Hence U.A.A. (%)	9.3	9.3	9.5	9.1
Flow-time at 25.0°C (water =200 secs.)	305.4 304.4	312.0 312.0	305.5 306.5	307.5 307.3
Hence $\frac{t-t_o}{t_o}$	0.52	0.56	0.53	0.54
$[\alpha]_D^{18°}$	+108°	+109°	+108°	+108°

Table continued on p53

Table - XIX (continued)

	S.S. 63					
	(a)	(b)	(c)	(d)	(e)	(f)
pH	2.80	2.90	2.89	2.90	2.90	2.83
Free reducing sugars (%)	0.16	0.13	0.07	0.35	0.20	0.10
Free titrable acidity (ml. 0.022N NaOH per 5 ml.)	4.20 4.18	3.48 3.48	3.68 3.64	3.62 3.64	3.63 3.66	3.65 3.68
Ml. of 1N NaOH per gm.	0.616	0.510	0.537	0.532	0.537	0.541
Hence apparent equivalent weight	1623	1961	1863	1880	1862	1848
Hence U.A.A. (%)	10.9	9.0	9.5	9.4	9.5	9.5
Flow-time at 25.0°C. (water = 200 secs.)	333.0 334.0	305.0 304.5	308.0 307.0	306.6 307.0	305.0 304.0	309.0 309.3
Hence $\frac{t-t_o}{t_o}$	0.67	0.53	0.54	0.52	0.53	0.55
$[\alpha]_D^{18^\circ}$	+54°	+107°	+108°	+106°	+106°	+106°

Table - XX

Absolute weights of neutral sugars in ten A. nilotica samples.

Sample	Wt. of gum taken (mg.)	Wt. of Rib. added (mg.)	Wt. of Rib. recovered (mg.)	Wt. of Gal. (mg.)	Wt. of Ara. (mg.)
62 a	94.9	14.6	12.6	41.3	43.7
62 b	99.5	14.3	12.4	43.2	46.8
62 c	114.2	16.3	14.4	47.3	52.6
62 d	107.1	10.4	9.4	46.2	49.8
63 a	94.6	17.3	15.4	31.1	51.9
63 b	83.4	13.3	11.8	33.2	38.8
63 c	81.8	16.0	14.0	35.0	37.0
63 d	86.5	17.5	16.0	36.5	40.5
63 e	94.7	14.0	12.0	41.0	43.1
63 f	83.9	15.2	13.6	32.9	41.1

Rib. = Ribose

Gal.= Galactose

Ara. = Arabinose

Table - XXI

Percent composition of ten A. nilotica gum samples.

Sample	U.A.A. (%)	D-Gal.(%)	L-Ara (%)
62 a	9.2	44	46
62 b	9.2	43	47
62 c	9.4	41	46
62 d	9.3	43	47
63 a	10.9	33	55
63 b	9.3	40	46
63 c	9.2	43	45
63 d	9.3	42	47
63 e	9.1	43	46
63 f	9.3	39	49

The galactose and arabinose percentages are rounded off to the nearest whole number.

Table - XXII

Molar ratio of neutral anhydro sugars in ten *A. nilotica* samples.

Sample	D-Gal.	L-Ara.
62 a	1	1.26
62 b	1	1.30
62 c	1.	1.33
62 d	1	1.29
63 a	1	2.00
63 b	1	1.40
63 c	1	1.26
63 d	1.	1.31
63 e	1	1.30
63 f	1	1.50

Fractionation of A. nilotica gum on diethylaminoethyl cellulose:

The method was fully described in Part- I. A trial experiment using 400 mg. of electrodialysed A. nilotica 62 a gave distinct fractions with 0.10M and 0.25M (major) phosphate buffer, and with 0.10M, 0.25M, and 0.50M sodium hydroxide. For subsequent step-wise fractionation only the above molarities were used. Using the same procedure, fractionation of A. nilotica 63a was attempted and similarly five fractions were obtained, the largest with 0.25M phosphate buffer. In all cases the elution patterns were similar as shown in Fig. (I - IV).

Each fraction was carefully dialysed in running water, further de-ionised on Amberlite IR 45(OH) and IR 120 (H), and finally taken to small volume and freeze-dried. Table (XXIII) shows the weights of the different fractions obtained.

Table - XXIII

	phosphate buffer		Aq. sodium hydroxide			Total wt. recovered (mg.)	Wt. taken (mg.)	Recovery
	0.10M	0.25M	0.10M	0.25M	0.50M			
(1)62a	23	206	62	16	14	321	400	80%
(2)62a	14	154	58	31	33	290	400	73%
(3)62a	44	342	30	26	12	454	500	91%
Total weight (mg.)	81	702	150	73	59	1065	1300	82%
(4)63a	12	105	93	48	20	278	300	93%
(5)63a	62	376	30	25	-	493	600	82%
Total weight (mg.)	74	481	123	73	20	771	900	86%

Results of the analytical analyses on the two major fractions are

shown below in Table (XXIV).

Table - XXIV

Fraction	$[\alpha]_D^{20}$	U.A.A. (%)	Methoxyl (%)	D-Gal. (%)	L-Ara. (%)	Flow-time 1% soln. (water = 186 secs)	Hence $\frac{t - t_o}{t_o}$
0.25M phosphate 62a	+108°	9.4	1.10	43	47	256 256	0.38
0.25M phosphate 63a	+58°	11.4	1.47	34	54	270 271	0.45

Hydrolysis of the 0.50M NaOH fraction gave the same component sugar residues as the parent gum. No xylose, which was thought to be obtained as a result of the degradation of the cellulose (79), was detected.

It is hoped that further work will be done to investigate more fully the heterogeneity of A. nilotica by (a) using larger columns of DEAE-cellulose and (b) employing another fractionation technique.

DISCUSSION

Of the sixteen A. senegal samples investigated (Tables V - IX), "hennawi" stands out as different in many respects. Its ash content (5.8%) is high while its rhamnose content (5.0%) is much lower than that of typical senegal (14%). The remaining fifteen samples show little or no variations at all in their composition and physical constants (U.A.A. : 14.5 ± 0.1 %, OMe : 0.35 ± 0.05 %, nitrogen : 0.34 ± 0.02 %, apparent equivalent weight: ca. 1400 and $[\alpha]_D^{20}$: -28 ± 1°). The small decrease in the nitrogen content of the purified samples suggests that some nitrogen is removed during electrodialysis. All samples were completely soluble in water to give almost colourless clear solution.

However, whether the apparent variation in the viscosities of the gum samples (Table VI) may prove to be structurally significant cannot be decided at this stage. In particular the samples obtained from trees growing on the sandy soil appear to give slightly more viscous solutions than those from trees growing on clay soil.

With the exception of "hennawi" these samples are similar in every respect and therefore it can be concluded that gum arabic obtained from the Sudan retains its characteristic physical properties irrespective of shape of nodule, season of picking or type of soil where the tree grows. Some support is lent to this conclusion from the finding that the examination of several samples of Virgilia oroboides gum taken at different seasons from a number of trees revealed that the samples were similar in respect of specific rotation and equivalent weight

(100).

Comparison of the properties and composition of the seven different Acacia gums analysed, reveals, like Acacia gums studied to-date, that they all contain D-galactose, L-arabinose, D-glucuronic acid and L-rhamnose (Table X). However, the proportions of these component residues are markedly different, and the ~~optical~~ specific rotations of these gums differ widely. Table XI shows that the uronic acid anhydride content ranges from 6.4 % (A. nubica) to 14.0 % ("hennawi"), the galactose content ranges from 28% (A. drepanolobium) to 52% (A. campylacantha) and the arabinose content varies from 26% (A. campylacantha) to 60% for A. nubica . Only two of the Acacia gums studied so far have given positive optical rotations, namely A. karoo (75) and A. seyal Del (79), to which must now be added five others, mamely, A. nilotica (+108°), A. arabica (+97°), A. drepanolobium (+74°), A. fistula (+60°) and A. nubica (+100°). The other two species, A. campylacantha (-3°) and A. dealbata (-24) are similar to A. senegal (-27°) in giving negative optical rotations. (Table XVII).

The marked variation in the proportions of the component sugar residues and the wide differences in the optical rotations of these gums must reflect differences in the detailed structures of the individual gums. In particular, the proportions of L-rhamnose in these gums differs considerably. It is present in trace quantity in A. fistula, A. arabica and A. nilotica while A. campylacantha has a rhamnose content of 7% (Table XI). It has been noticed that the uronic acid/rhamnose ratio is very nearly unity for some gums e.g. gum arabic 14.5/14, mollisima gum 9/7, campylacantha 8.2/7.0 and dealbata 7.7/6.0. This may

mean that rhamnose is linked glycosidically to the acid residues in these gums (101, 102). On the other hand, the rhamnose content of a few gums is so low that it may possibly be present in an adherent impurity and not a main structural feature of the gum.

Both A. nilotica and A.arabica have a rhamnose content of 0.4% which could only be estimated by a micro infra-red method (99) and no rhamnose could be detected by the usual chromatographic methods.

Again the results shown in Tables XIV and XVI show marked differences in the methoxyl content of the Acacia gums. The values range from 0.12% for A. nubica to 1.44% for A. nilotica (63 a). These results indicate that the presence of methoxyl groups in Acacia gums is a more general occurrence than hitherto believed. Methoxyl groups occur frequently in plant gums as 4-O-methyl-D-glucuronic acid (e.g., in Albizzia (103) and Khaya (104) species) or as ester groups (e.g., Sterculia (105) and Astragalus (106) gums). The suggestion that the methoxyl content of Acacia gums may be of structural significance (83) is now given support by the isolation of 4-O-methyl-D-Glucuronic acid from A. nilotica (Part III). In the same gum this acid has been found to be a component of two different aldobiouronic acids.

In earlier investigation of plant gums, the nitrogen content was either not investigated (109, 107, 108) or was recorded without comment (101, 100). The nitrogen contents of the seven Acacia gums investigated are widely different; the values range from 0.02% for A. nilotica to 1.13% for A. drepanolobium (Table XVI). Apart from the small decrease in the nitrogen

content during electrodialysis, the removal of nitrogen from plant gums is difficult. Similar difficulties regarding the complete removal of nitrogen exist in investigation of starch and glycogen (111) and it has been suggested (112) that this nitrogen is present in residual traces of the enzyme system involved in biosynthesis. Investigation of the nitrogen content of the Acacia gums may well prove to be of value in throwing light on the origin and mode of formation of these gums.

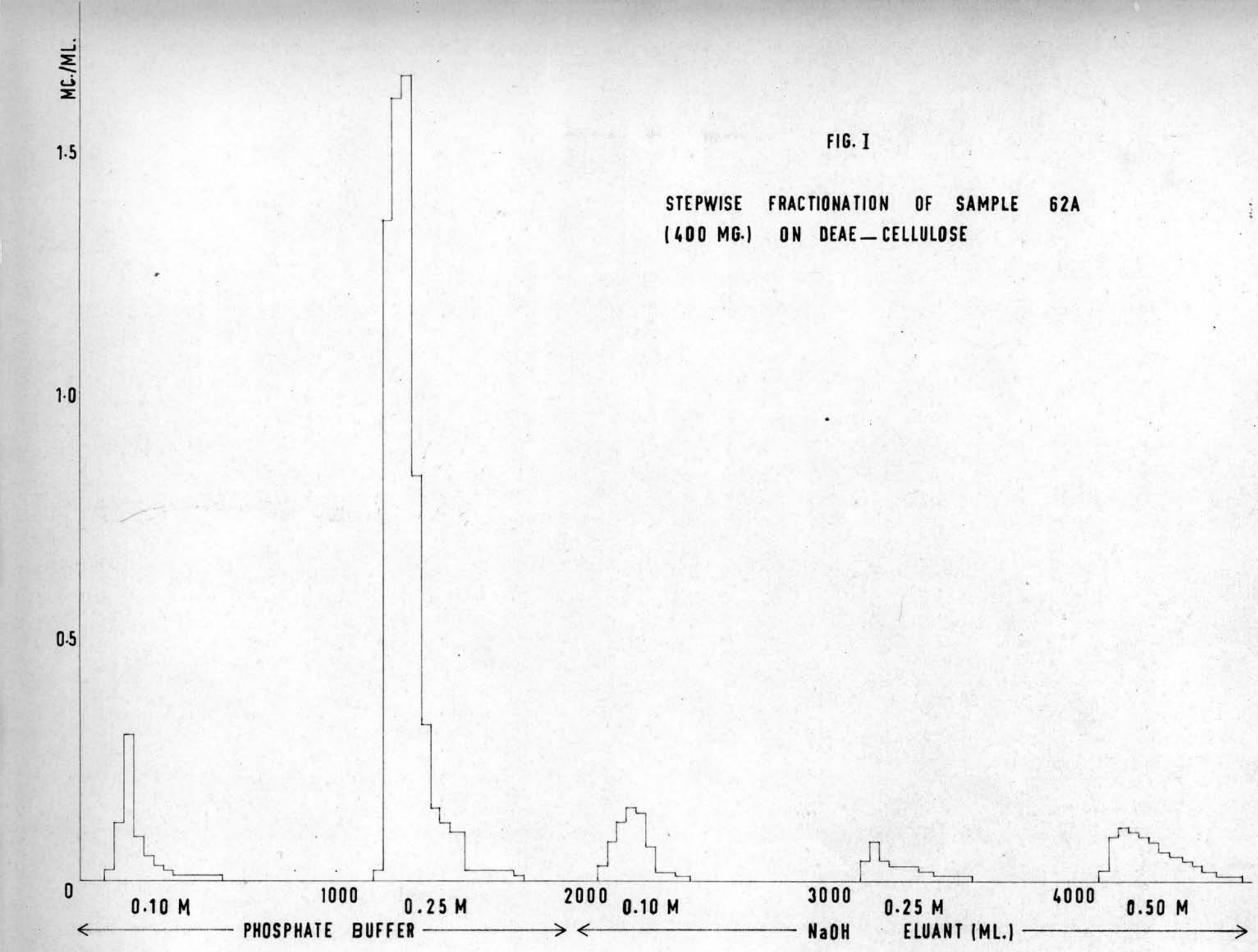
With the exception of A. nilotica sample 63a, all the other A. nilotica specimens studied have similar ~~optical~~ specific rotations ($+107^{\circ} \pm 1^{\circ}$), equivalent weights (1912 ± 50), viscosity coefficients and composition (U.A.A. 9.3 ± 0.1%; galactose 42 ± 2% ; arabinose 47 ± 2%; Tables XVIII - XXII). However the apparent similarity disappears when consideration is given to the methoxyl content (0.75% to 1.44% ; Table XVIII), which has been shown in these studies to be structurally significant by the isolation of 4-O-methyl-D-glucuronic acid. Since attempts to establish alternative or additional sites of methoxyl groups (e.g., as a methylated sugar or as ester groups) have failed, it is believed that the proportion of this acid varies with the proportion of methoxyl in the various specimens.

Previous studies in this field have shown that internodule variations exist (e.g., in the gums from Combretum leonense (26), and A. seyal Del (79)). In both investigations the single nodules differed markedly in their composition. Other studies, however, point in the opposite direction. An examination of several samples of Virgilia oroboides gum taken at different seasons from a number of trees showed that they were similar in

respect of specific rotation and equivalent weight (100). Also the essential homogeneity of the gum ~~Carapa~~ Capera procera (113) was confirmed by the insignificance of the differences in the constants obtained for purified specimens from different trees. Furthermore, the results (Tables XIV - XVII) from preliminary investigation of three A. nubica samples show that no significant variation occurs in the composition and the physical constants of these samples (U.A.A. 6.2 ± 0.2%; OMe 0.12%; nitrogen 0.20%; $[\alpha]_D$ +99± 1°).

Despite its submission by the Sudanese collector as such, specimen 63a cannot be considered to be a specimen of A. nilotica in view of its anomalous behaviour. It differs widely from the remaining nine A. nilotica samples in its ~~optical~~ specific rotation (+54°; other nine samples +108°), methoxyl and uronic acid anhydride contents, viscosity, and galactose and arabinose contents. The values of these analytical parameters (Tables XVII - XXII) suggest that specimen 63a is much more similar to A. fistula than to A. nilotica.

Studies of the behaviour of A. nilotica gum on DEAE-cellulose showed that only one major fraction could be obtained, and that with o.25M phosphate buffer. However fractionation of gum arabic and other Acacia gums (114) on DEAE-cellulose invariably gave two main fractions: one with phosphate buffer (approx. 0.25M) and one with sodium hydroxide. However, in view of the accumulating evidence indicating the heterogeneity of plant gums, the homogeneity of A. nilotica cannot be confirmed on the basis of the data available at present.


MC./ML.
1·5
1·0
0·5
0
FIG. I
STEPWISE FRACTIONATION OF SAMPLE 62A
(400 MG.) ON DEAE — CELLULOSE
0·10 M
1000
0·25 M
2000
0·10 M
3000
0·25 M
4000
0·50 M
PHOSPHATE BUFFER
NaOH
ELUANT (ML.)

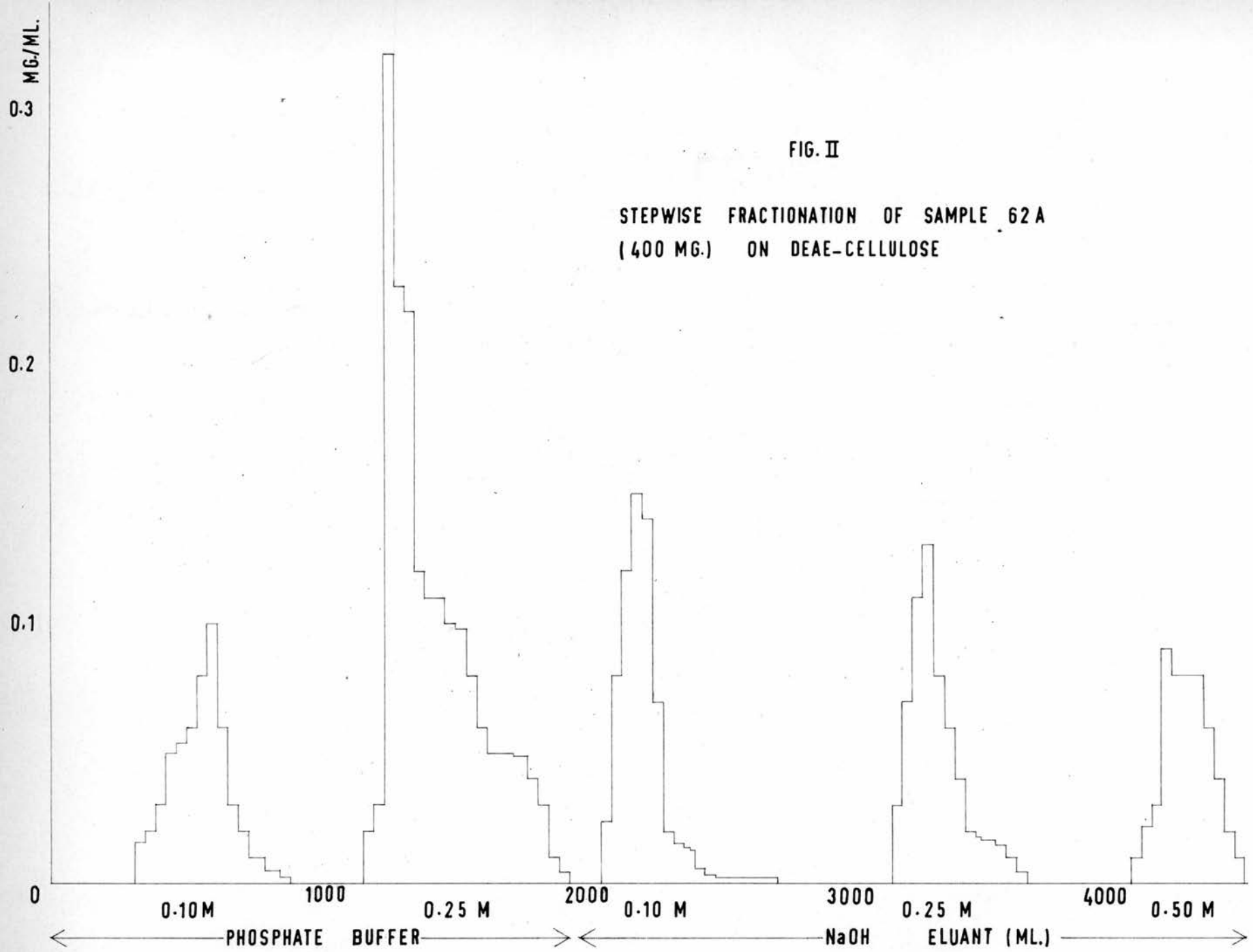


FIG. II

STEPWISE FRACTIONATION OF SAMPLE 62 A
(400 MG.) ON DEAE-CELLULOSE

FIG. III

STEPWISE FRACTIONATION OF SAMPLE 62A
(500 MG.) ON DEAE—CELLULOSE

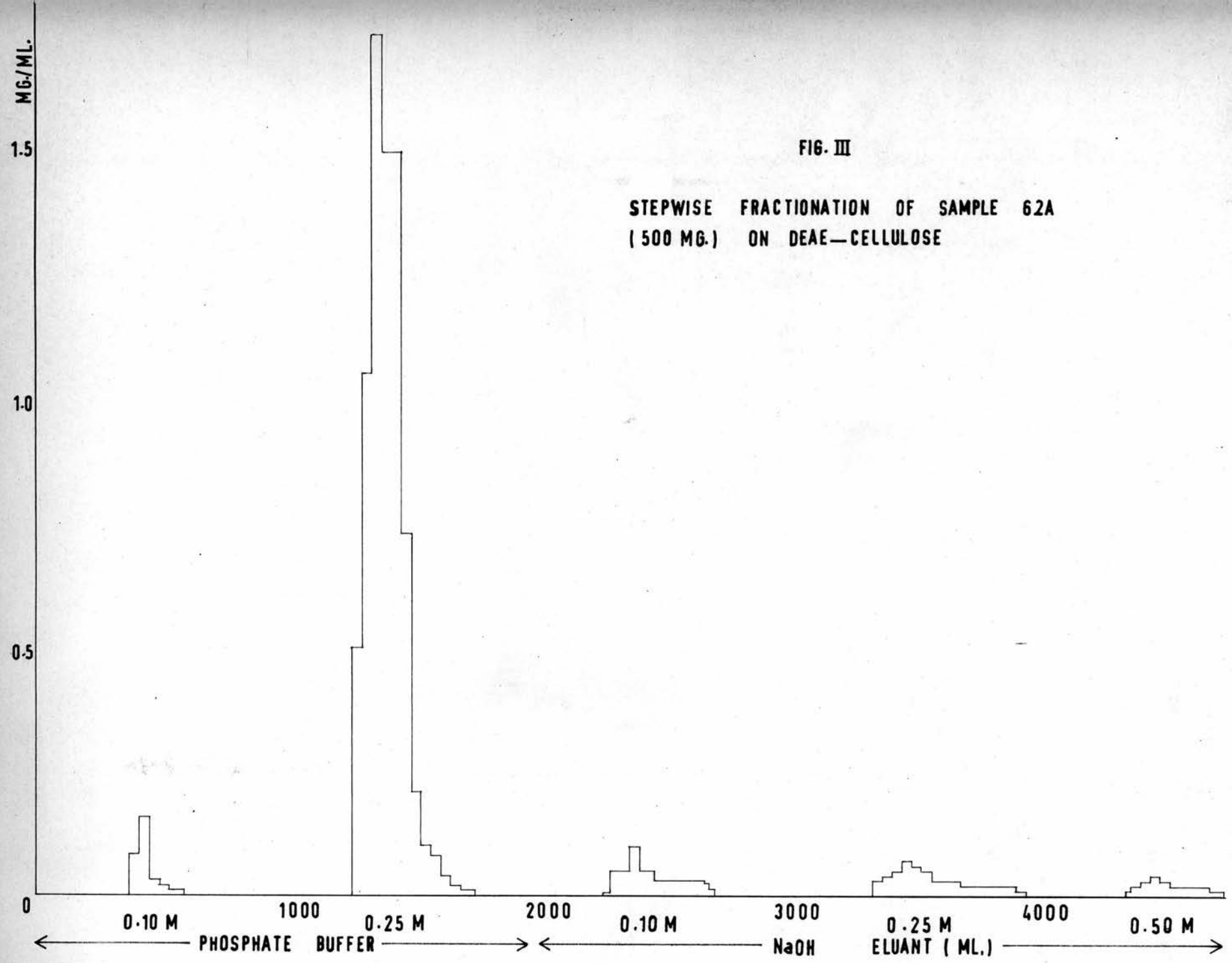


FIG. IV

STEPWISE FRACTIONATION OF SAMPLE 63A
(300 MG.) ON DEAE—CELLULOSE

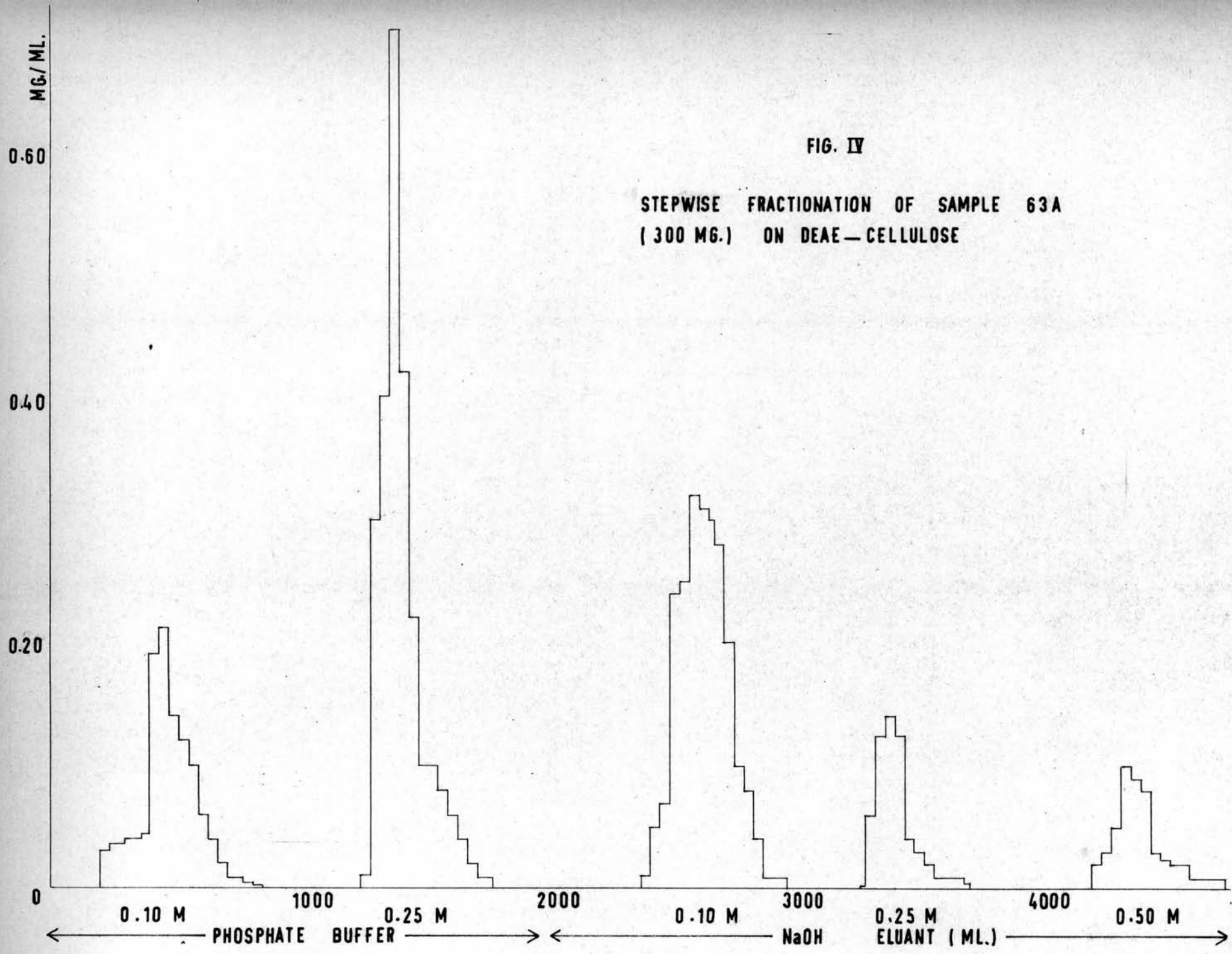


FIG. V

STEPWISE FRACTIONATION OF SAMPLE 63A (600 MG.) ON DEAE — CELLULOSE

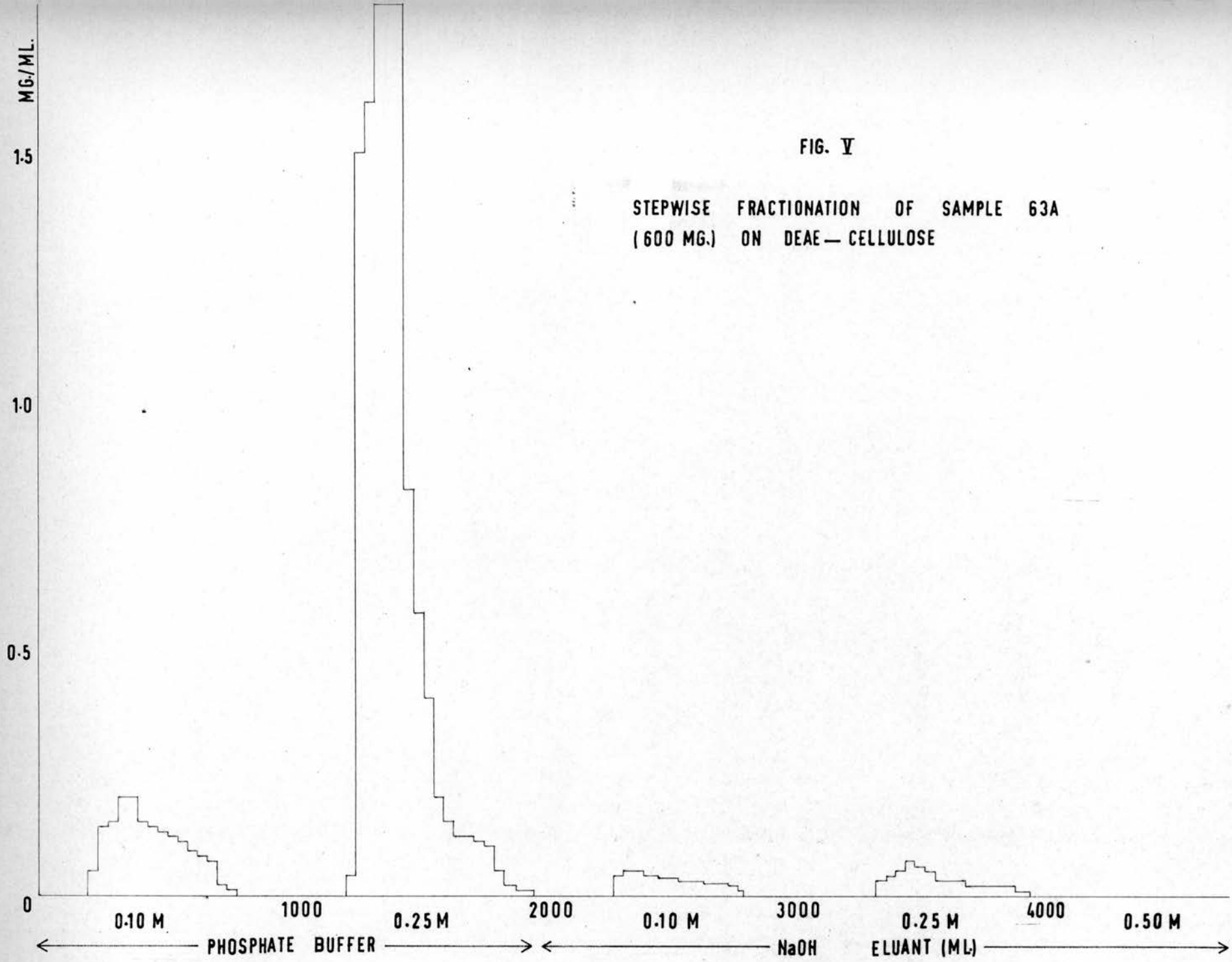
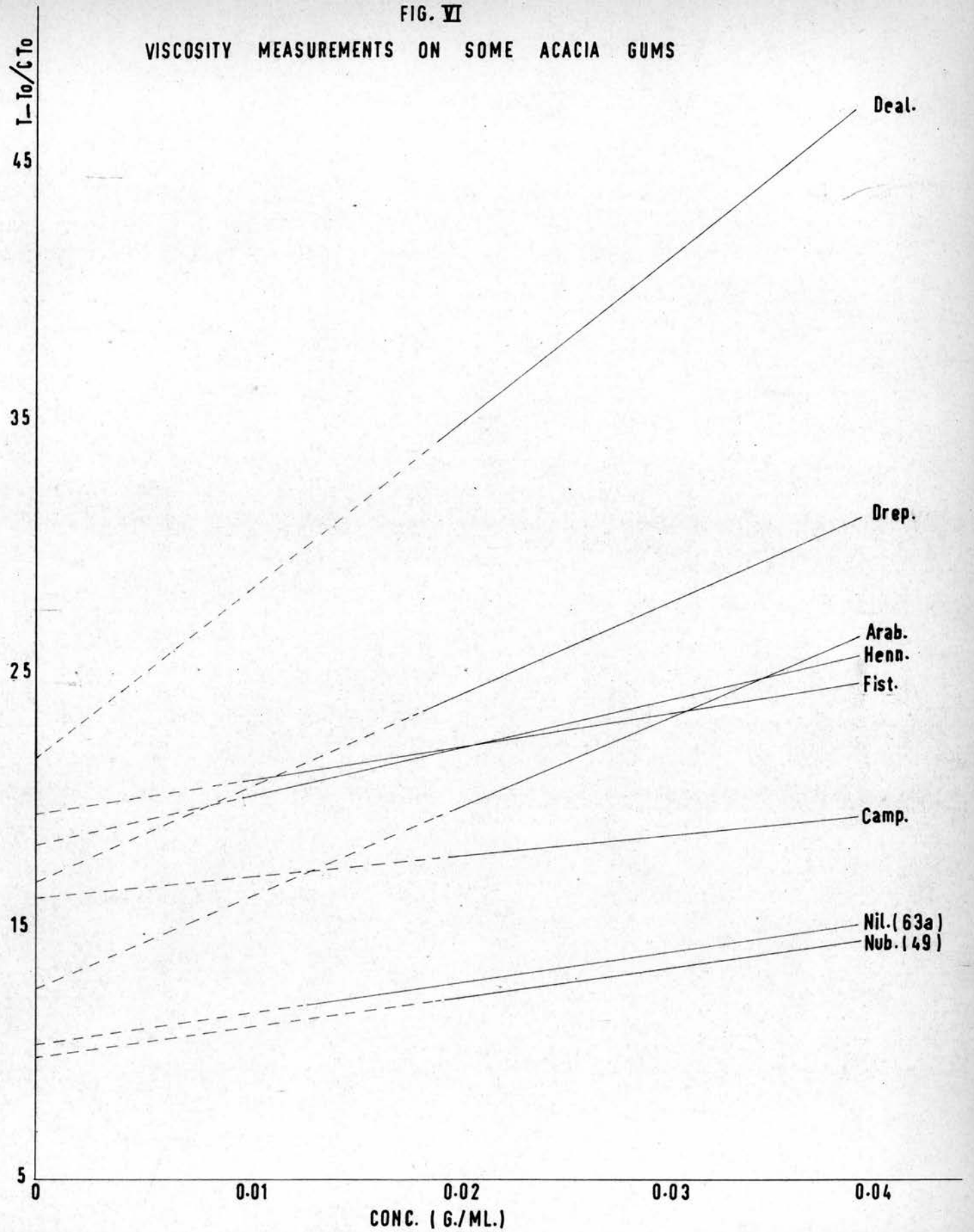


FIG. VI

VISCOSITY MEASUREMENTS ON SOME ACACIA GUMS



PART III

STRUCTURAL INVESTIGATIONS ON THE GUM EXUDATE FROM ACACIA NILOTICA

PARTIAL HYDROLYSIS STUDIES

(A) AUTOHYDROLYSIS

(i) Trial Experiment: A solution of the electrodialysed gum (sample SS 62A; 3 g. in 100 ml. water) was heated on a boiling water bath for 60 hours. The reaction was followed both polarimetrically and chromatographically. Aliquots (2 ml.) were withdrawn at intervals for determination of optical rotation and identification of the sugars liberated, using Whatman No.4 paper run in solvent B.

Time	$[\alpha]_D^{20^\circ}$	L-Ara	Arabinobiose	D-Gal
0	+108.0°	-	-	-
1	+108.5°	+	trace	-
2	+109.0°	+	trace	-
6	+112.0°	+	+	-
15	+115.0°	+	+	-
20	+116°	+	+	-
36	+117°	+	+	trace
60	+117°	+	+	trace

After 60 hours the solution was cooled, and dialysed in distilled water for 72 hours whereby most of the liberated sugars were recovered as a golden syrup. Yield of dry syrup A=1.5g. The degraded gum was precipitated from the solution with ethanol acidified with a few drops of hydrochloric acid, centrifuged and washed several times with ethanol. The degraded gum was then dissolved in water and freeze-dried. Yield of degraded gum (A) = 0.4g. About 20 mg. of degraded gum A was hydrolysed with 4 ml. 2N H_2SO_4 for 8 hours and the sugar ratio determined by the phenol sulphuric acid method (28). Found L-Ara : D-Gal = 1:3 (cf. found for whole gum L-Ara:D-Gal= 4:3)

(ii) Large Scale Autohydrolysis: 9g. of the gum acid were dissolved in 300 ml. of water and the solution was heated on a boiling water bath for 24 hours after which time the solution was cooled and treated in the same way as before to give 5.2 g. of golden syrup B, and 3.6 g. of degraded gum B. The degraded gum had U.A.A. 10.2%, and number average molecular weight of 33,000 ± 5,000 (99). The degraded gum B was retained for graded hydrolysis studies.

The syrup (5.2 g.) on chromatographic examination in solvents B and C showed only two spots; these gave a pink colouration with aniline oxalate, and corresponded to L-arabinose and 3-O-β-L-arabopyranosyl-L-arabinose. The syrup was dissolved in a small amount of water and absorbed in a charcoal-celite column (30 X 5 cm.).

The column was first developed with water (5L.) to elute most of the arabinose, then with gradually increasing concentration of aqueous alcohol. The aqueous fraction on evaporation to dryness gave a thick crystalline syrup (4.2 g.) which, on examination chromatographically in solvents A and B showed only one spot corresponding to L-arabinose. This syrup, on recrystallisation from methanol, gave crystalline L-arabinose $[\alpha]_D^{20}$ +105° (c, 2.0 in water), m.p. and mixed m.p. 159°C. The aqueous ethanol fraction (0.7 g.) on chromatographic examination in solvent B showed one strong spot corresponding to the neutral disaccharide mentioned above, and a weak spot corresponding to L-arabinose. It was separated into two fractions using thick paper in solvent B:-

Fraction I : Crystalline L-arabinose

Yield = 0.14 g. $[\alpha]_D^{20}$ + 105° (c, 1.0 in water).

m.p. and mixed m.p. 159° after recrystallisation

from methanol.

Fraction II: 3-O-β-L-arabinopyranosyl-L-arabinose

Yield = 0.54 g. $[\alpha]_D^{20}$ +199° (c 1.0 in water).

This fraction was chromatographically pure and identical to 3-O-β-L-arabinopyranosyl-L-arabinose and having R_{Gal} 0.80 and 0.68 in solvents B & C, and D respectively. Attempts to recrystallise the partially crystalline sugar were not successful. Hydrolysis of a small amount of the sugar with 0.5N hydrochloric acid at 100°C for 4 hours furnished only L-arabinose. The phenylosazone was prepared, and, on recrystallisation from ethanol, gave yellow crystals having m.p. 233° (with decomposition; lit. m.p. 235°).

A portion (200 mg.) of the partially crystalline syrup was methylated by Kuhn's method (57). Hydrolysis with 1N hydrochloric acid at 100°C and neutralisation with silver carbonate gave two spots with R_G values 0.78 and 0.62 in solvent A. The methylated product (140 mg.) was fractionated on the thick paper using solvent A. Two distinct fractions identical chromatographically to 2,3,4-tri-O-methyl-L-arabinose and 2,4-di-O-methyl-L-arabinose were obtained.

Fraction I: 2,3,4-tri-O-methyl-L-arabinose

yield = 58 mg. $[\alpha]_D^{20}$ +144 (c, 1.0 in water).

R_G 0.78 in solvent A. The amide of the aldonic acid was prepared and on recrystallisation from absolute ethanol had m.p. 105° (lit. 107°) and $[\alpha]_D^{20}$ +24° (c= 0.25 in water).

Fraction II: 2,4-di-O-methyl-L-arabinose

Yield= 49 mg. $[\alpha]_D^{20}$ +118° (c= 0.2 in water).

R_G 0.62 in solvent A. The aniline derivative on recrystallisation from ethyl acetate had m.p. 128° (lit. 126°).

(B) PARTIAL HYDROLYSIS (I) - Isolation of Neutral Disaccharides:

Degraded gum B (0.5 g.) was dissolved in 0.5N sulphuric acid (25 ml.) and the solution was heated under reflux on a boiling water bath. Aliquots (2 ml.) were removed at varying intervals of time (½ hr. 1 hr., 1½ hr., 2 hr., 3 hrs., 4 hrs., 5 hrs., 6 hrs.,) and the contents examined chromatographically in solvents B and D. Galactose and arabinose were present in all the aliquots. Furthermore, visual inspection of the resulting chromatograms suggested that the best yield of neutral disaccharides could be obtained by hydrolysis of the degraded gum with 0.5N sulphuric acid for 1½ hours at 100°.

Accordingly, the degraded gum B (2 g.) was hydrolysed with 0.5N sulphuric acid (100 ml.) for 1½ hours at 100°. After cooling, the solution was partly neutralised with solid barium hydroxide and the neutralisation was completed by the addition of solid barium carbonate. The resulting solution was centrifuged, and the filtrate and washings concentrated to ca. 20 ml. and poured into ethanol (80 ml.) to precipitate any undegraded gum. The precipitate was removed by centrifugation and washed with ethanol (3 X 25 ml.) and dried. Yield of syrup C = 0.15 g. After dissolving in water and de-ionising with IR-120 resin, this was examined chromatographically in solvent D and was found to contain mainly acid mono- and disaccharides and was not examined further. The supernatant liquid containing neutral mono- and disaccharides was concentrated to syrup D. Yield = 1.6g.

Separation of Neutral Disaccharides

The above syrup (D), on examination chromatographically (52 hours) in solvent D, showed, in addition to galactose and arabinose, four spots having R_{Gal} values o.66 (faint spot; later identified as a mixture of acid disaccharides), 0.39 (heavy spot), 0.28 (faint spot) and 0.21 (faint spot; later identified as a mixture of acid disaccharides). However, examination of syrup D in solvent system B, showed in addition to arabinose and galactose, one heavy spot (R_{Gal} 0.51 corresponding to 3-O-β-D-galactopyranosyl-D-galactose), a faint spot (R_{Gal} 0.28 corresponding to 6-O-β-D- galactopyranosyl-D-galactose) and a faint streak probably due to acid disaccharides. The syrup was therefore separated on thick paper in solvent B (72 hours) and the fractions with R_{Gal} values 0.51 and 0.28 isolated.

Fraction I: 3-O-β-D-galactopyranosyl-D-galactose.

R_{Gal} 0.51 in solvent. Yield = 158 mg.

$[\alpha]_D^{20}$ + 57° (c, 1.0 in water).

This fraction was isolated as a partially crystalline syrup. Needles, m.p. and mixed m.p. 154° were obtained on treatment with aqueous ethanol. The syrup gave single spot having the same mobility as 3-O-β-D-galactopyranosyl-D-galactose in solvents B, C and D. Only galactose was obtained on hydrolysis with 1N sulphuric acid for 5 hours.

A portion (12 mg.) was oxidisedwith sodium periodate in the dark at room temperature. Oxidation was allowed to proceed over-night and corresponded to the consumption of 1.94 mole of periodate for one mole of disaccharide.

Methylation of sugar (80 mg.) followed by methanolysis and

hydrolysis, gave two methylated sugars. These were separated on 3MM paper using solvent A when the respective R_G values were 0.88 and 0.70.

Fraction Ia

This fraction (18 mg.) was chromatographically pure and identical to 2,3,4,6-tetra-O-methyl-D-galactose which was identified as its aniline derivative (m.p. and mixed m.p. 190°).

Fraction Ib

This fraction (16 mg.) was chromatographically pure and identical to 2,4,6-tri-O-methyl-D-galactose. The aniline derivative had m.p. 165° (lit. 165°).

Fraction II

(33 mg.) 6-O- β -D-galactosyl-D-galactose

This fraction was isolated as a syrup having $[\alpha]_D^{20}$ + 28° (c, 0.3 in water) and was chromatographically pure and identical to 6-O- β -D-galactosyl-D-galactose in solvents B,C and D. Chromatography of the acid hydrolysate gave a single spot corresponding to galactose. No methylation studies were attempted because of the small amount of the disaccharide available.

(C) HYDROLYSIS OF THE POLYSACCHARIDE, AND ISOLATION OF THE ACIDIC FRACTION

The electrolydialysed polysaccharide (16 g.; U.A.A. 9.2%; methoxyl = 0.96%) was hydrolysed at 100° with 1N sulphuric acid (300 ml.) for 12 hours, after which time the solution was allowed to cool and then filtered off. The pale yellow solution was heutralised with barium hydroxide and barium carbonate, centrifuged, evaporated to small volume, passed through a column of Amberlite resin IR - 120 (H) inorder to remove barium ions, and evaporated to a syrup which, on examination by paper chromatography in solvents (B) and (D), was shown to contain D-galactose, L-arabinose, D-glucuronic acid, 4-O-methyl-D-glucuronic acid, and a mixture of acid disaccharides (two spots).

The separation of neutral sugars from acidic components was attempted using (a) a column of Amberlite CG 45 (OH), and (b) Duolite A-4, in the formate form. A more successful separation of neutral and acidic components of the hydrolysate was achieved on a column of the weakly basic Duolite A-4 resin. The syrup was placed on the column (4X 36 cm.), the neutral sugars eluted with copper-free water, and the column washed with copper-free water, till the washings gave negative test with phenol-sulphuric acid. Yield of syrup of neutral sugars (D-galactose and L-arabinose only) = 10.7 g. and this syrup was not examined further. The acidic fraction was washed off the resin column with 5% formic acid (1 litre), and evaporated to small volume. Yield of syrup = 4.4 g.

Fractionation of the Acidic Mixture on a Cellulose Column:

The cellulose column (4 X 80 cm.) was first thoroughly

washed with water and then with one litre of ethylacetate-acetic acid-water ((9:2:2 ; v/v). The acidic fraction (4.4 g.) of the gum hydrolysate was absorbed on to circles of thick filter paper placed on the column, and covered by a further inch of cellulose powder. The column was allowed to stand overnight before eluting with solvent (E) and the contents of every third tube (10 ml.) were examined in solvents D and E. Five fraction were obtained in all. Each fraction was shaken with an equal volume of water, and the organic layer discarded. The aqueous layer was evaporated to small volume, shaken with ether in order to remove the rest of the ethyl acetate and acetic acid, and evaporated to dryness. The different fractions obtained are shown in the table below. The recovery from the column was 89%.

Table

Fraction	Wt. (in mg.)	Constituent sugars
I (tubes 24 to 28)	216	D-glucurone
II (tubes 30- 61)	1498	4-O-methyl-D-glucuronic acid
III (tubes 61 - 100)	931	D-glucuronic acid (mainly) D-galactose (trace).
IV (tubes 134 - 307)	941	4-O-α(4-O-methyl-α-glucuronosyl)-galactose and 6-O-(4-O-methyl-β-glucuronosyl)-galactose
V (tubes 492 - 600)	309	6-O-β-glucuronosyl-galactose

Examination of the Fractions:

Fraction I. D-glucurone (216 mg.). $R_{Gal.}$ 3.0 in solvent E. Crystallised out readily. After recrystallisation from water had m.p. and mixed m.p. 177° $[\alpha]_D^{20}$ +19 (c 1.0 const.).

Fraction II. 4-O-methyl-D-glucuronic acid (1.498 g.) Found OMe, 14.6. Calculated for mono-O-methylhexuronic acid: OMe, 14.9%. This gave a single yellow-brown spot, moving just before rhamnose (R_{Rh} = 0.91) in solvent D. This fraction had R_{Gal} 0.39 in solvent B, and $[\alpha]_D^{20}$ +37° (c, 1.5 in water).

The golden syrup (0.4 g.) was left overnight, at room temperature, in contact with dry methanolic solution of hydrogen chloride (4 %; 50 ml) in sealed tubes, then heated for 8 hours on a boiling water bath, cooled and neutralised with silver carbonate. The silver chloride was filtered off and washed repeatedly with hot methanol. The methyl ester methyl glycoside was isolated on evaporation of the combined filtrate and washings (0.34 g.). The partially crystalline syrup was dissolved in water (50 ml.) and added slowly to a solution of potassium borohydride (0.25 g. in 25 ml. of water) and left overnight. Excess hydride was destroyed by the addition of dilute acetic acid. The solution was then de-ionised with Amberlite resin IR- 120 (H) to remove potassium ions. It was then reduced in volume and evaporated several times with methanol to remove borate ions. The methyl glycoside (0.29 g.) obtained was hydrolysed for 2 hours in 1N hydrochloric acid to give a product (0.24 g.) running on paper just slower than L-rhamnose in solvent A (R_{Rh} = 0.94). The product was further purified on 3 MM paper in solvent A. On

further examination in solvents B and D, the product gave a single spot having R_{Gal} values of 1.94 and 2.1 respectively. (OMe = 15.0 %; calc. for $C_7H_{14}O_6$: OMe 15.6 %). The m.p. of crystalline orange phenylosazone derivative was 56° (lit. 57 - 60°) after two recrystallisations from boiling water.

Preparation of the Amide of 4-O-methyl-D-glucuronic acid.

0.2 g. of the acid was dissolved in dry methanolic hydrogen chloride (2 %; 20 ml.) and left overnight in a sealed tube at room temperature. Then it was heated in a boiling water bath for 8 hours, cooled and neutralised with silver carbonate. The solution was filtered and the residue extracted with hot methanol and acetone. The filtrate and washings were concentrated to a syrup (0.17 g.). The syrup (methyl ester methyl glycoside of the acid) was treated with saturated methanolic ammonia solution (15 ml.) and left for a week at 0°C. Evaporation afforded a syrup which rapidly crystallised. Recrystallisation from absolute ethanol twice gave large colourless plates of the amide of the methyl-4-O-methyl-D-glucuronoside (138 mg.), m.p. 231° (lit. 232 - 236°); $[\alpha]_D^{20}$ +140° (c= 0.5 in water).

Fraction III. (0.931 g.) D-glucuronic acid plus trace of galactose. This fraction gave a single spot moving at the same rate as D-glucuronic acid in solvent D. In solvent B, however, another spot (faint) was observed which corresponded to D-galactose run as control. As D-glucurone was already characterised above, and since D-glucuronic acid and D-glucurone exist in equilibrium in solution, this fraction was not examined further.

Fraction IV. Aldobiouronic acids mixture A:

Yield = 0.941 g. $[\alpha]_D^{19}$ + 55° (c, 1.0 in water).

Chromatographic examination of the partially crystalline syrup showed a brown spot with aniline oxalate having R_{Gal} values of 0.66 and 0.69 in solvents E and D respectively. Hydrolysis with 2N sulphuric acid for 6 hours and subsequent examination by paper chromatography in solvent D gave D-galactose and 4-O-methyl-D-glucuronic acid (R_{Rh} 0.98) in equal proportions (visual examination).

Reduction of the Mixture of Aldobiouronic Acids

A small portion (80 mg.) of the acid mixture was subjected to methanolysis (3% methanolic hydrogen chloride; 10 ml.) for 8 hours. The solution was then neutralised with silver carbonate, filtered and evaporated to dryness. The syrup was dissolved in water (5 ml.) and was added dropwise to a 1% solution of potassium borohydride (25 ml.). After 18 hours the excess of potassium borohydride was destroyed with dilute acetic acid and the solution was de-ionised with Amberlite resin IR-120 (H). Borate ions were removed by several evaporations with methanol. The residue was hydrolysed on a boiling water bath with 1N sulphuric acid for 15 hours. The hydrolysate after neutralisation and deionisation was examined by paper chromatography in solvents A and B where two spots were observed in each case. One spot (the slower moving) corresponded to D-galactose run as control, while the other (4-O-methyl-D-glucose) had R_{Rh} values of 0.94 and 0.90 in solvents A and B respectively. The reduced product was fractionated on 3MM paper in solvent A to give two fractions:-

Fraction IV (a) 4-O-methyl-D-glucose

(28 mg.) $[\alpha]_D^{20}$ +58° (c, 0.1 in water)

It was converted to the phenylosazone and on recrystallisation from aqueous ethanol orange crystals were obtained m.p. 158° (lit. 159°).

Fraction IV (b) Crystalline D-galactose

(31 mg.) $R_{Gal.}$ 1.0 in solvent B

This fraction on recrystallisation from absolute ethanol had m.p. and mixed m.p. 156°.

Methylation of Aldobiouronic Acids Mixture A

A sample (500 mg.) of the mixture A was dissolved in water (8 ml.) and 1 ml. each of dimethyl sulphate and 30% aqueous sodium hydroxide solution was added through 2 hours. The reaction mixture was kept in an ice bath and the solution stirred vigorously (magnetic stirrer) in an atmosphere of nitrogen. Aliquots of dimethyl sulphate (10 ml.) and 30% aqueous sodium hydroxide solution (20 ml.) were added over 3 days. Then the mixture was heated on boiling water bath for 30 minutes to destroy unreacted dimethyl sulphate, acidified with dilute sulphuric acid till just acidic and the sodium sulphate precipitated with methylated spirits. The solution was filtered and the residue extracted with more methylated spirits. The filtrate and extract were concentrated to small volume (ca. 30 ml.) after being made slightly alkaline. This solution was then made acidic (2 ml. of 2N H_2SO_4) and extracted with chloroform (5 X 100 ml.) in the cold. The chloroform extracts were concentrated, filtered and the partly methylated product precipitated by addition of light petroleum (b.p.

60 - 80°). The partly methylated product (386 mg.) was methylated to completion with methyl iodide (20 ml.) and silver oxide (8 g.) in dry methanol (10 ml.), the silver oxide added over a period of 8 hours. The mixture was then filtered and the residue extracted continuously with boiling chloroform overnight. The extract and filtrate were evaporated to a dry syrup (0.32 g.). A further Purdie methylation was carried out on the syrup to give methylated derivative (286 mg.) having OMe of 52.1%. Further Purdie methylation did not increase the methoxyl content significantly.

Reduction of the Methylated Aldobiouronic Acids Mixture A

Powdered lithium aluminium hydride (150 mg.) was added to the solution of the syrup (286 mg.) in dry tetrahydrofuran (50 ml.), amdthe mixture was refluxed gently for 2½ hours, a further 50 mg. of lithium aluminium hydride being added after the first hour. Excess lithium aluminium hydride was then destroyed by the careful addition of water and ethyl acetate, the hydroxide precipitates were filtered off and extracted repeatedly with dry acetone and dry chloroform. The extracts and the mother liquor were combined and evaporated to dryness. The syrup was re-extracted with dry chloroform, filtered and filtrate evaporated to dryness to give the methylated neutral product (213 mg.)

Hydrolysis of 8 mg. of syrup with 1N hydrochloric acid for 4 hours and subsequent chromatographic examination of the products gave 3 spots with R_G values 0.84, 0.72 and 0.69 in solvent A. The bulk of the methylated product was therefore hydrolysed in the same way. Since two of the methylated sugars ran so close together on paper, attempts to separate them ~~on~~ by paper

chromatography were not very successful. A satisfactory separation was achieved using a small cellulose column (2 X 38 cm.), employing as solvent butan-1-ol (70%) -light petroleum (b.p. 100 -120°) (30%)- saturated with water. Fractions were obtained as follows:

(i) 2,3,4-tri-O-methyl-D-glucose (68 mg.)

R_G = 0.85 in solvent A.

This was chromatographically pure and identical to 2,3,4-tri-O-methyl-D-glucose in solvent A,B, and F and was finally characterised by the preparation of the aniline derivative, which after recrystallisation from ethyl acetate had m.p. 152° (lit. 145 - 150°).

(ii) 2,3,6-tri-O-methyl-D-galactose (18 mg.)

R_G = 0.71 in solvent A.

Chromatographic examination of this fraction showed one spot with the same mobility as 2,3,6-tri-O-methyl-D-galactose in solvents A and F. The product was oxidised with Br_2 to 2,3,6-tri-O-methyl-galactolactone, which on treatment with ether gave long fine needles melting at 96-7° (lit. 98°).

(iii) 2,3,4-tri-Omethyl-D-galactose (17 mg.)

R_G= 0.68 in solvent A.

This was partially crystalline and chromatographic examination in solvents A,B and F showed one spot running at the same rate as 2,3,4-tri-O-methyl-D-galactose run as control. Finally the aniline derivative was prepared and after recrystallization from ethyl acetate had m.p. and mixed m.p. 166° (lit. 167 - 170°). Finally the column was eluted with water and on chromatographic examination of this fraction no sugar could be detected.

Separation of Aldobiouronic Acids Mixture A on 3MM Paper

Attempts were made to see if mixture A could be separated into its constituents. This was first considered to be a mixture of aldobiouronic acids as a result of the observation that the R_{Gal} values and $[\alpha]_D$, obtained for the corresponding fraction IV resulting from partial acid-hydrolysis of different A. nilotica gum specimens, varied significantly. In particular $[\alpha]_D$ varied from +48° to +71°. This was not surprising as both aldobiouronic acids later isolated from this fraction had one methoxyl group and it was seen earlier (Part II) that A. nilotica specimens varied significantly in their methoxyl content. Separation of fraction IV (mixture A) into two distinct aldobiouronic acids was achieved satisfactorily using narrow strips (4 inches wide) of 3MM paper in solvent D for 96 hours. Chromatograms were freed from acid solvent by drying in air for 48 hours followed by heating for 5 minutes at 150°. The appropriate strips were eluted with cold water to give the following two fractions:

Fraction IV (a)

4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose. (Fig. IV, p. 83) Yield = 101 mg. $[\alpha]_D^{20}$ +93° (c, 1.0 in water). R_{Gal} 0.79 in solvent A.

The high specific rotation indicates an α linkage.

A sample (40 mg.) was converted into the methyl ester methyl glycoside. Half of the latter was reduced with potassium borohydride. The derived syrup gave on hydrolysis equal amounts (visual inspection) of galactose and 4-O-methyl-D-glucose (R_{Rh} 0.94 in solvent A). The other half of the methyl ester methyl glycoside was subjected to periodate oxidation for 24 hours

(½% sodium metaperiodate solution) and was observed to reduce 1.8 moles of sodium periodate per mole of glycoside.

A sample (8mg.) of the aldobiouronic acid was dissolved in 2 ml. of water and sodium bicarbonate buffer (2 ml.) added together with 4 ml. of ½% sodium metaperiodate solution. The mixture was left in the dark for 24 hours to ensure that reaction was complete. 1 ml. aliquots were withdrawn and the amount of formaldehyde present estimated using a standard curve for formaldehyde prepared by the periodate oxidation of D-glucose. It was found that 1.01 mole of formaldehyde was liberated per mole of acid disaccharide. The experiment was repeated on 10 mg. of the aldobiouronic acid and it was observed that 1.04 mole of formaldehyde was liberated per mole of acid disaccharide . This indicated that the C_6 in the galactose moiety of the molecule was not involved in linkage.

Fraction IV (b)

6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose (Fig. II P. 83). Yield = 95 mg. $[\alpha]_D^{20}$ +6° (c, 0.95 in water). R_{Gal} = 0.68 in solvent D.

Methanolysis followed by potassium borohydride reduction and subsequent hydrolysis with mineral acid gave galactose and 4-O-methyl-D-glucose (R_{Rh}= 0.88) only as shown by paper chromatography in solvent D.

The methyl ester glycoside derivative of the aldobiouronic acid was treated with 0.5 percent sodium periodate and kept at room temperature in the dark for 2 days. Periodate consumed at this stage was found to be 3.1 moles per mole of disaccharide. Furthermore no formaldehyde could be detected after oxidation of a small portion of this acid disaccharide at pH 8.

Fraction V, Aldobiouronic Acid Mixture B

Mainly 6-O-(-β-D-glucopyranosyluronic acid)-D-galactose plus a trace of 4-O-(α-D-glucopyranosyluronic acid)-D-galactose.

Yield = 309 mg. $[\alpha]_D^{20}$ +31° (c=1.2 in water)

Hydrolysis furnished only D-galactose and D-glucuronic acid as revealed by paper chromatography in solvents B and D. Methanolysis followed by potassium borohydride reduction and subsequent hydrolysis with mineral acid furnished D-galactose and D-glucose. The latter must have arisen from the reduction of D-glucuronic acid. The R_{Gal} value (0.24 in solvent D) and the relatively high positive ~~optical~~ specific rotation suggested this night be a mixture of two aldobiouronic acids (lit. $[\alpha]_D$ for 6-O-(-β-D-glucopyranosyluronic acid)-D-galactose is ca. -5°). The syrup was examined chromatographically in solvent D on No. 1 paper for 120 hours. Two distinct spots were observed: one strong spot, R_{Gal} 0.21, corresponding to 6-O-(-β-D-glucopyranosyluronic acid)-D-galactose, the other (faint spot), R_{Gal} 0.28. The syrup was subsequently fractionated in 3MM paper in solvent D, using narrow strips (4 inches wide) and good separation was obtained after developing the narrow chromatograms for 160 hours. The sugars were isolated in the same manner as for fraction IV above.

Fraction V a.

6-O-(-β-D-glucopyranosyluronic acid)-D-galactose. (Fig. I P. 83). Yield = 103 mg. $[\alpha]_D^{20}$ -5.0 (c, 0.4 in water). R_{Gal} 0.21 in solvent D.

This fraction was chromatographically homogeneous, giving a single spot having the same mobility as authentic 6-O-(-β-D-glucopyranosyluronic acid)-D-galactose in solvents D, E and F.

Methanolysis followed by potassium borohydride reduction and subsequent hydrolysis gave only D-galactose and D-glucose. The latter must have arisen from the reduction of D-glucuronic acid. The low negative ~~optical~~ specific rotation of the fraction (-5°) indicated a β linkage.

Methylation of a portion (80 mg.) by Haworth's and Purdie's methods followed by reduction with lithium aluminium hydride, afforded the methylated neutral product (38 mg.). Hydrolysis with mineral acid afforded two methylated products with R_G values 0.85 and 0.67 in solvent A. These were separated on 3MM paper using solvent A to give:

(a) 2,3,4-tri-O-methyl-D-glucose (14 mg.)

R_G = 0.85 in solvent A.

The aniline derivative was prepared and after recrystallisation from ethyl acetate had m.p. 148° (lit. 145 - 150°).

(b) 2,3,4-tri-O-methyl-D-galactose (11 mg.)

R_G = 0.67 in solvent A.

This fraction was chromatographically pure, and was identical to 2,3,4-tri-O-methyl-D-galactose run as control in solvents A, D and F. The aniline derivative was prepared, but attempts to recover crystals from the syrup were not successful.

Fraction V (b)

4-O-(-α-D-glucopyranosyluronic acid)-D-galactose

(Fig. III, P. 83). Yield (18 mg.) $[\alpha]_D^{21}$ +107° (c, 0.1 in water). R_{Gal} 0.28 and 0.32 in solvent D on Whatman No. 1 and 3MM paper respectively.

Hydrolysis of 4 mg. fraction with 2N sulphuric acid for 5 hours in sealed tube furnished D-galactose and D-glucuronic acid only. A sample (8 mg.) was subjected to periodate oxidation at pH 8 for 24 hours. A definite release of formaldehyde was observed

(0.93 mole per mole of a 2,3 or 4-linked acid disaccharide). The high ~~optical~~ specific rotation (+107°) would indicate an α -linkage. Fig. III, P.83 gives one of the possible structures, of this acid disaccharide on the evidence available above. Owing to the trace quantity of acid disaccharide available no methylation studies were attempted.

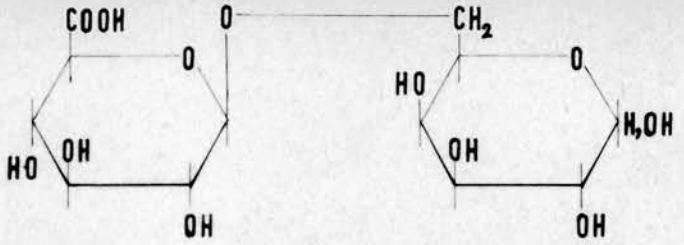


FIG. I

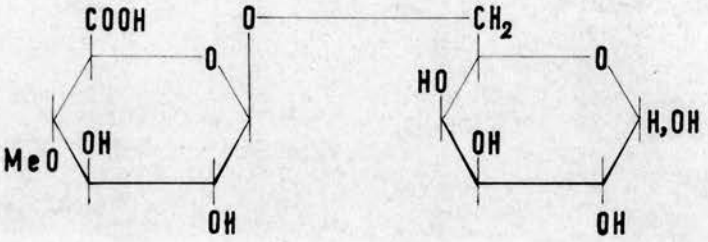


FIG. II

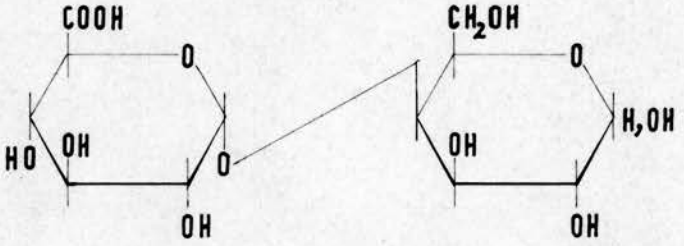


FIG. III

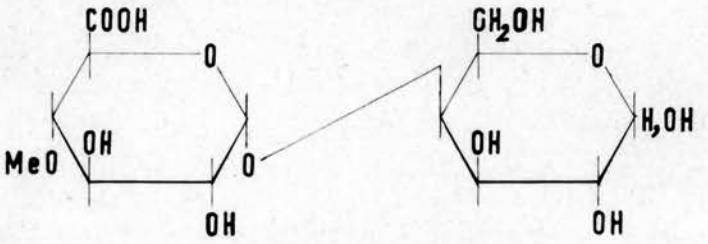


FIG. IV

Methylation of Whole Gum

The gum acid (15 g.) was dissolved in water (80 ml.) and methylated in an atmosphere of nitrogen below 20°C, in a five litre flask. Methyl sulphate (250 ml.) and sodium hydroxide solution (30%, 500 ml.) were added dropwise with stirring over a period of 12 hours; stirring was continued overnight (12 hours). Two further additions of the same quantities of the reagents were made.

After the third methylation there resulted a separation of the sodium salt of partly methylated gum into yellow, pliable crystalline masses. These were mechanically separated, dissolved in water, acidified with 5N sulphuric acid, whereupon the partly methylated gum acid precipitated as a white powder. The latter was then taken up in chloroform, dried over anhydrous sodium sulphate, and the solution concentrated to small volume under vacuum. When the concentrated chloroform solution was poured into excess of light petroleum (b.p. 60 - 80°) with stirring, the methylated gum acid was obtained as a white amorphous powder (10.2 g.; %OMe 25.1). A further addition of the methylated reagent to the supernatant liquid did not result in the isolation of any further methylated product.

Methylation was continued using aqueous acetone (50% v/v; 200 ml.) as the solvent for the partly methylated gum acid. Methyl sulphate (250 ml.) and aqueous sodium hydroxide (30%; 600 ml.) were added over a period of ten hours and the stirring continued overnight. After two further additions of the same quantities of the reagents, the methylation was completed by heating the solution for half an hour at 60°C, whereby the excess

of acetone was expelled and excess of dimethyl sulphate ~~destroyed~~ expelled. Immediately the sodium salt of the methylated gum began to separate on the surface of the methylation mixture as insoluble, pale yellow masses as described above. These were filtered and purified as described above. (4.0g.; %OMe 37.6). No more crystals appeared after a further addition of methylating reagents to the supernatant liquor.

A seventh methylation of the gum acid gave 2.6 g. of methylated acid with OMe of 40.1%.

The methylated gum was dissolved in methanol (20 ml.) and methyl iodide (20 ml.). The mixture was refluxed gently in a dry flask fitted with a water condenser and calcium chloride guard tube. Silver oxide (8 g.) was added in four batches every three hours. The residue was filtered and extracted several times with boiling chloroform. The combined filtrate and extract were concentrated to a small volume and the methylated gum was precipitated by the addition of excess light petroleum (b.p. 60 - 80°). Yield of product 2.4 g.; OMe 40.4 %.

A small amount (400 mg.) of the methylated gum was subjected to a further Purdie methylation, but as the % OMe value did not increase significantly, the main bulk of the methylated gum (2.0g.) was taken as fully methylated.

As the yield appeared to be rather poor, an attempt was made to carry out six methylations successively without isolating the product over a period of 6 days. 15 g. of gum acid (SS 63b) was methylated by the procedure above and this resulted in a higher yield (7.01 g.) but a lower OMe % value (36.5 %). The results of methylation are summarised in the table below.

(H = Haworth; P = Purdie)

Sample	Methylation No.	% OMe	Yield (g)
62 a (15 g.)	0	0.96	15.0
	3 H	25.1	10.2
	6 H	37.6	4.0
	7 H	40.1	2.6
	8 P	40.4	2.4
63 b (15 g.)	0	1.3	15.0
	6 H	36.5	7.01
	8 P	40.2	5.9
	9 P	40.2	5.5

Hydrolysis of the Methylated Gum

A trial hydrolysis was carried out on 100 mg. each of the methylated gum specimens (SS 62 a, 63 b). The methylated gum was heated with methanolic hydrogen chloride (4%, 10 ml.) for 8 hours in a sealed tube on a boiling water bath, neutralised with silver carbonate, filtered and the filtrate evaporated to dryness. A small amount of the syrup was examined by vapour-phase chromatography, when the presence of at least eight different methylated sugars was detected (Table XXV). Both methylated gums produced the same methanolysis products. The remainder of the syrup was subjected to a further hydrolysis with 1N sulphuric acid for 3 hours. Examination by paper chromatography in solvents A and C confirmed the presence of the methylated sugars indicated by

vapour phase chromatography.

T a b l e XXV

Methanolysis Products of Methylated Whole Gum as Detected by Vapour-phase Chromatography.

Methyl glycoside of	T values in system ( )	
2,3,5-tri-O-methyl-L-arabinose	0.55 & 0.71(a)	0.48 &0.60(b)
2,5-di-O-methyl-L-arabinose	1.83 & 3.40(a)	0.70 & 1.04(b)
2,3,4-tri-O-methyl-L-arabinose	1.04 (a)	0.82 (b)
2,3-di-O-methyl-L-arabinose ?	1.56(a)	0.96 (b)
2,3,4-tri-O-methylD-glucuronic acid	2.45 & 3.08(a)	1.72 & 2.16(b)
2,4,6-tri-O-methyl-D-galactose	3.93 & 4.40(a)	2.03 & 2.35(b)
2,3,4-tri-O-methyl-D-galactose	6.93 (a)	2.54 & 2.67(b)
2,4-di-O-methyl-D-galactose		3.52 & 4.17(b)
2,3,4,6-tetra-O-methyl-D-galactose		1.60 (b)

(a) = Butanediolsuccinate polyester (175°)

(b) = Polyphenol (200°)

T = Retention time of the methyl ester methyl glycoside relative to that of methyl-2,3,4,6-tetra-O-methyl-β-D-glucopyranoside.

The bulk of the methylated whole gum (2 g.; $[\alpha]_D^{20}$ ± 71±2°; (c,1in $CHCl_3$)) was refluxed in 5% methanolic hydrogen chloride (50 ml) for 24 hours. The solution was neutralised with silver carbonate, filtered and evaporated to give syrup of methyl glycosides. To saponify the methyl esters of the acidic components, the syrup was dissolved in barium hydroxide solution (50 ml.,

saturated at room temperature) and heated for two hours at 60°. The solution was then filtered and passed through a column of IR 120 (H) and the resin washed until sugar-free.

A column of purified and regenerated Duolite A-4 resin was prepared and the effluent from the cation exchange resin passed through. The acidic methylated sugars were selectively removed and the neutral sugars eluted with water (2 litres). The eluate and washings were evaporated carefully to a syrup A.

The acidic component was isolated from the resin column by displacement with N sodium hydroxide (250 ml.) and the eluate passed several times through a column of IR 120 (H) to remove cations. The eluate and washings were evaporated to give a syrup B (yield 0.158 g.).

The mixture of methyl glycosides of neutral sugars (syrup A) was heated in N hydrochloric acid (30 ml.) at 100°C for five hours. Then the solution was neutralised with silver carbonate, filtered and the residue extracted with hot methanol. The filtrate and washings were combined and on evaporation gave a syrup containing the neutral methylated sugars. Yield = 1.81 g.

Fractionation of the Neutral Methylated Sugars:

The neutral methylated sugars (1.81) were separated by chromatography on cellulose column (4 x 80 cm.) The column was eluted successively with the following solvents:

(a) Light petroleum (b.p. 100 - 120°) : butan-1-ol (3:7, saturated with water)

(b) Light petroleum (b.p. 100 - 120°): butan-1-ol (1:1

saturated with water).

(c) Butan-1-ol half saturated with water.

(d) Water

The fractions were obtained (Table XXVI, p 90) and the recovery was 86%.

Examination of the Fractions:

Fraction I. (41 mg.) 2,3,5-tri-O-methyl-L-arabinose

Paper chromatography of this fraction, $[\alpha]_D^{20}$ -39° (c= in water), R_G= 0.98 in solvent A, showed it to be homogeneous and identical to 2,3,5-tri-O-methyl-L-arabinose. With aniline oxalate spray it gave a characteristic black stain (red in U.V. light). Demethylation gave only arabinose. Finally it was characterised by conversion into the crystalline amide of the aldonic acid (2,3,5-tri-O-methyl-L-arabonamide), which on recrystallisation twice from ethyl acetate, had m.p. 135° (lit. 130 - 138°).

Fraction II (196 mg.) 2,3,4,6-tetra-O-methyl-D-galactose .

This fraction, R_G 0.88 in solvent A, $[\alpha]_D^{20}$ +101° (c, 1.0) was chromatographically identical to 2,3,4,6-tetra-O-methyl-D-galactose in solvents A and C. On demethylation only galactose was obtained. The sugar was finally characterised by preparing the aniline derivative (2,3,4,6-tetra-O-methyl-N-phenyl-D-galactosylamine), which after recrystallisaion from ethanol, had m.p. and mixed m.p. 194 - 5° (with authentic sample, m.p. 195°).

Fraction III (211 mg.) mainly 2,5-di-O-methyl-L-arabinose plus trace of 2,3,4-tri-O-methyl-L-arabinose.

This fraction on chromatography in solvent A showed one elongated spot which was black but which was pink-brown at the top. However, chromatography in solvent C gave two distinct spots,

Table - XXVI

Neutral methylated sugars from whole gum.

Fraction	R_G	Colour with aniline oxalate	Wt. (mg.)	Contents
1	0.98	Black, red in U.V.	41	2,3,5-tri-O-methyl-L-arabinose
2	0.88	Reddish-brown	196	2,3,4,6-tetra-O-methyl-D-galactose
3	0.80	Black red in U.V.	211	2,5-di-O-methyl-L-arabinose
	0.78	Pink-brown		2,3,4-tri-O-methyl-L-arabinose
4	0.78	Pink-brown	164	2,3,4-tri-O-methyl-L-arabinose
	0.75	Reddish-brown		2,4,6-tri-O-methyl-D-galactose
5	0.75	Reddish-brown	66	2,4,6-tri-O-methyl-D-galactose
6	0.70	Reddish-brown	157	2,3,4-tri-O-methyl-D-galactose
7	0.59	Pink-brown	21	2,3-di-O-methyl-L-arabinose
8	0.53	Reddish-brown	778	2,4-di-O-methyl-D-galactose
9	0.53	Reddish-brown	23	2,4-di-O-methyl-D-galactose
10	0.22	Reddish-pink streak	26	Acidic methylated sugars (trace).

corresponding to 2,5-di-O-methyl-L-arabinose (black spot) and 2,3,4-tri-O-methyl-L-arabinose (pink spot). The syrup was separated into two fractions on thick paper using solvent C.

Subfraction (a), R_G 0.80 in solvent A and $[\alpha]_D^{20}$ -2° (c, 0.5 in water). On chromatographic examination in solvents A, B and C, this fraction (96 mg.) was homogeneous and identical to 2,5-di-O-methyl-L-arabinose; it gave the black colouration with aniline oxalate usually associated with the furanose ring form. On preparation of the amide of the aldonic acid and subsequent recrystallisation from ethyl acetate, a crystalline product was obtained which had m.p. 127°C undepressed on admixture with an authentic sample of 2,5-di-O-methyl-L-arabonamide. On demethylation only arabinose was obtained.

Subfraction (b), R_G 0.76 and $[\alpha]_D^{20}$ +118° (equilib.) (c=0.8 in water). This fraction (80 mg.) was chromatographically identical to 2,3,4-tri-O-methyl-L-arabinose, and gave a pink colouration with aniline oxalate which suggested that the sugar was in the pyranose ring form. Demethylation gave arabinose only. Finally the sugar was characterised by the preparation of the amide of the aldonic acid which had m.p. 106 - 7° (lit. m.p. 107°).

Fraction IV, (164 mg.) mainly 2,4,6-tri-O-methyl-D-galactose plus trace of 2,3,4-tri-O-methyl-L-arabinose.

Chromatography in solvent C gave two distinct spots: one pink and the other reddish-brown on spraying with aniline oxalate. The syrup was separated on thick paper in solvent C.

Subfraction (a) (11 mg.) R_G 0.76 in solvent A. This fraction was chromatographically identical to 2,3,4-tri-O-methyl-L-arabinose and gave a pink colouration with aniline oxalate which suggested that the sugar was in the pyranose ring form. Demethylation gave

arabinose only. Attempts to prepare the amide of the aldonic acid as described for subfraction (b) of fraction III above, failed because of the small quantity of the methylated sugar available.

Subfraction (b) R_G 0.75 in solvent A and $[\alpha]_D^{18°}$ +86° (c=1.0 in water) (equil). This fraction (142 mg.) was chromatographically pure and identical to 2,4,6-tri-O-methyl-D-galactose. The aniline derivative (2,4,6-tri-O-methyl-N-phenyl-D-galactosylamine) gave needle shaped crystals when recrystallised from acetone:ether: petroleum (1:1:1) and which had m.p. 165° (lit. 165°). On demethylation only galactose was obtained.

Fraction V, 2,4,6-tri-O-methyl-D-galactose.

R_G 0.75 (solvent A). $[\alpha]_D^{20}$ +86° (equil) (c,0.5 in water). Chromatographic examination of this fraction (66 mg.) in solvents A, B, and C revealed only one spot, which was identical to 2,4,6-tri-O-methyl-D-galactose. The aniline derivative was obtained with m.p. 165° (lit. 165°). On demethylation only galactose was obtained.

Fraction VI 2,3,4-tri-O-methyl-D-galactose

R_G 0.70 (solvent A). $[\alpha]_D^{20}$ +110° (c, 0.5 in water).

This fraction (157 mg.), when examined chromatographically was pure and corresponded to 2,3,4-tri-O-methyl-D-galactose in solvents A and B. On demethylation only galactose was obtained. It was shown to be 2,3,4-tri-O-methyl-D-galactose by conversion into 2,3,4-tri-O-methyl-N-phenyl-D-galactosylamine, m.p. 168° (lit. 167 - 170°).

Fraction VII R_G 0,60 (solvent A) $[\alpha]_D^{20}$ +80° (c, o.25 in water). This fraction (21 mg.) gave a single spot in solvent A and C corresponding to 2,3,di-O-methyl-L-arabinose. The amide of the aldonic acid, however, failed to crystallise. On

demethylation only arabinose was obtained.

Fraction VIII 2,4-di-O-methyl-D-galactose (778 mg.)

R_G 0.53 (solvent A) $[\alpha]_D^{20}$ +105° +90°(equil)

(c,1.0 in water).

Examination of this fraction in solvents A, B and C showed only one spot corresponding to 2,4-di-O-methyl-D-galactose. The sugar readily crystallised on standing at room temperature overnight, and on recrystallisaion from aq. acetone, colourless crystals were obtained, m.p. 96°C, which gave on admixture with authentic specimen of 2,4-di-O-methyl-D-galactose monohydrate, did not undergo depression. The sugar was observed to be resistant to periodate oxidation. Demethylation gave only galactose.

Fraction IX (23 mg.) R_G 0.53 (solvent A).

This fraction was easily crystallised and chromatographically was identical to 2,4-di-O-methyl-D-galactose. Recrystallisation from acetone furnished colourless crystals which had m.p. 97° undepressed on admixture with authentic 2,4-di-O-methyl-D-galactose.

Fraction X R_G 0.22 (solvent A).

This fraction (26 mg.) which was obviously an acidic component was obtained on eluting the cellulose column with water. It gave a reddish-pink streak in solvent A and C. It was therefore combined with syrup B (acidic methylated sugars).

Examination of the Acidic Methylated Sugars:

Syrup B (158 mg.) was combined with fraction X above and refluxed with 2% methanolic hydrogen chloride (20 ml.) for 4 hours, neutralised with silver carbonate and filtered. The residue was extracted with hot methanol and the filtrate and the extracts concentrated to a syrup (138 mg.). The product was dissolved in anhydrous tetrahydrofuran (20 ml.), lithium aluminium hydride (100 mg.) was added, and the mixture was refluxed gently for one hour. More lithium aluminium hydride (50 mg.) was then added and the mixture was refluxed for another hour. Excess lithium aluminium hydride was removed by the cautious addition of water and ethyl acetate alternately. The mixture was filtered, the residue washed with hot acetone and chloroform, and the combined filtrate and washings taken to dryness. The product was then extracted with dry chloroform, filtered, and the filtrate taken to dryness again. This reduced material was finally hydrolysed with 1N hydrochloric acid (15 ml.) for 3 hours, the solution neutralised with silver carbonate, deionised, and concentrated to a syrup (118 mg.). Paper chromatography in solvents A and C showed that the syrup contained only one neutral methylated sugar corresponding to 2,3,4-tri-O-methyl-D-glucose. This compound therefore must have arisen from the reduction of D-glucuronic acid (and 4-O-methyl-D-glucuronic acid) originally present in the gum acid. The product had $[\alpha]_D^{20}$ +76° (c, 0.8 in water) and R_G 0.83 in solvent A. The methylated sugar was finally identified by the preparation of the aniline derivative which gave a m.p. of 130°, and which, when mixed with an authentic sample of 2,3,4-tri-O-methyl-N-phenyl-D-glucosylamine, was not depressed.

METHYLATION OF THE DEGRADED GUM:

The degraded gum B (U.A.A. 10.2%) prepared by the autohydrolysis of the A. nilotica gum, was converted into its fully methylated derivative (OMe 36.8%) according to the procedure already described for the methylation of the whole gum. To a solution of the barium salt of the degraded gum (4 g.) in water (20 ml), dimethyl sulphate (25 ml.) was added slowly. A 30 percent solution of sodium hydroxide (50 ml.) was added during 8 hours and the reaction mixture stirred for 12 hours. Five further additions of the reagents were made. The reaction product was isolated in the manner described for the methylation of the whole gum. This was methylated twice with Purdie's reagents (yield 1,51 g.; OMe 36.8%). Further methylation of this product with Purdie's reagents did not increase its methoxyl contents.

Hydrolysis of the Fully Methylated Degraded Gum:

The methylated degraded gum (1.51 g.) was dissolved in methanolic hydrogen chloride (6%; 50 ml.) and refluxed for 24 hours. The solution was neutralised with silver carbonate, filtered and the filtrate evaporated to give a syrup of methyl glycosides which on examination by gas chromatography revealed the presence of at least eight methylated sugars (Table XXVII).

The mixture of the methyl glycosides was heated in 1N hydrochloric acid (25 ml.) at 100° for 6 hours. The solution was neutralised with silver carbonate, filtered and the residue extracted with hot methanol. The filtrate and washings were combined to give on evaporation a syrup (1,2 g.) The syrup (1 g.) was separated into eight fractions on cellulose column (4 X 80 cm.).

The column was eluted successively with the following solvents:-

(a). Light petroleum (b.p. 100 -120°) : butan-1-ol (7:3 saturated with water).

(b) Light petroleum (b.p. 100 - 120°) : butan-1-ol (1:1 saturated with water).

(c). Butan-1-ol half saturated with water.

(d). Water.

The results of fractionation are summarised in Table- XXVIII. Recovery = 88.5%.

Table - XXVII

Methanolysis products of methylated degraded gum as detected by vapour-phase chromatography.

Methyl glycoside of	T values in system ( )	
2,3,5-tri-O-methyl-L-arabinose	0.54 X 0.69(a)	0.46 X 0.62(b)
2,3,4-tri-O-methyl-L-arabinose	1.02 (a)	0.83 (b)
2,3,4,6-tetra-O-methyl-D-galactose	1.73 (a)	1.57 (b)
2,5-di-O-methyl-L-arabinose	2.00 ∝ 3.32(a)	0.60 ∝ 1.08 (b)
2,3,4-tri-O-methyl-D-glucuronic acid	2.41 ∝ 3.06(a)	1.72 ∝ 2.16(b)
2,4,6-tri-O-methyl-D-galactose	3.95 ∝ 4.44 (a)	2.03 ∝ 2.32(b)
2,3,4-tri-O-methyl-D-galactose	6.93 (a)	2.54 ∝ 2.80(b)
2,4-di-O-methyl-D-galactose		3.61 ∝ 4.19(b)

Table - XXVIII

Methylated sugars from the hydrolysis of methylated degraded gum.

Fraction	R_G	Colour with aniline oxalate	weight (mg.)	Contents
1	0.98	Black, red in U.V.	14	2,3,5-tri-O-methyl-L-arabinose
2	0.89	Reddish-brown	64	2,3,4,6-tetra-O-methyl-D-galactose
3	0.80	Black, red in U.V.	136	2,5-di-O-methyl-L-arabinose
	0.77	Pink-brown		2,3,4-tri-O-methyl-L-arabinose
4	0.73	Reddish-brown	39	2,4,6-tri-O-methyl-D-galactose
5	0.73	Reddish-brown	58	2,4,6-tri-O-methyl-D-galactose
	0.70	Reddish-brown		2,3,4-tri-O-methyl-D-galactose
6	0.70	Reddish-brown	144	2,3,4-tri-O-methyl-D-galactose
7	0.53	Reddish-brown	236	2,4-di-O-methyl-D-galactose
8 (water)	0.23	Reddish-pink (streak)	194	2,3,4-tri-O-methyl-D-glucuronic acid

Examination of the Fractions:

Fraction I, (14 mg.) 2,3,5-tri-O-methyl-L-arabinose

Paper chromatography of this fraction, $[\alpha]_D^{20}$ -40° (c,0.1 in water), R_G = 0.98 in solvent A, showed it to be homogeneous and identical to 2,3,5-tri-O-methyl-L-arabinose. With aniline oxalate spray it gave a characteristic black stain (red under U.V. light). Demethylation gave only arabinose. Attempts to prepare the amide of the aldonic acid ~~were~~ were not successful because of the small amount of fraction available for investigation.

Fraction II, (64 mg.) 2,3,4,6-tetra-O-methyl-D-galactose.

This fraction, R_G 0.88 in solvent A, $[\alpha]_D^{20}$ +100° (c, 0.5 in water), was chromatographically identical to 2,3,4,6-tetra-O-methyl-D-galactose in solventsA and C. On demethylation only galactose was obtained. The sugar was finally characterised by preparing the aniline derivative which, after recrystallisation from ethanol, had m.p. and mixed m.p. 195° (authentic sample m.p. 195°).

Fraction III, (136 mg.) mainly 2,5-di-O-methyl-L-arabinose
plus trace of 2,3,4-tri-O-methyl-L-arabinose.

This fraction on chromatography in solvent A showed one elongated spot mainly black but pink at the top. However chromatography in solvent C gave two distinct spots, corresponding to 2,5-di-O-methyl-L-arabinose (heavy spot) and 2,3,4-tri-O-methyl-L-arabinose (faint spot). The syrup was separated into two fractions on thick paper using solvent C.

Subfraction(a), R_G 0.80 in solvent A and $[\alpha]_D^{20}$ -2° (c,0.8 in water). On chromatographic examination in solvents A and C this fraction (110 mg.) was found homogeneous and identical to 2,5-di-O-methyl-L-arabinose, giving the black colouration with aniline oxalate

which is usually associated with the furanose ring form. On demethylation only arabinose was obtained. On preparation of the amide of the aldomic acid and subsequent recrystallisation from ethanol, a crystalline product was obtained which had m.p. and mixed m.p. 130°.

Subfraction (b), R_G 0.76 (8 mg.).

This fraction was chromatographically identical to 2,3,4-tri-O-methyl-L-arabinose and gave a pink colouration with aniline oxalate which suggested that the sugar was in the pyranose ring form. Demethylation gave only arabinose.

Fraction IV, (39 mg.) 2,4,6-tri-O-methyl-D-galactose.

This fraction had R_G 0.75 in solvent A and $[\alpha]_D^{20}$ +85° (c,0.3 in water). The aniline derivative was prepared and on recrystallisation from acetone:ether:petroleum (1:1:1) gave needle-shaped crystals which had m.p. 164° (lit 165°). On demethylation only galactose was obtained.

Fraction V, (58½ mg.) Mainly 2,4,6-tri-O-methyl-D-galactose plus trace of 2,3,4-tri-O-methyl-D-galactose. This fraction had $[\alpha]_D^{20}$ +90° (equilibrium) (c = 0.5 in water). The main component was characterised as the aniline derivative, which on recrystallisation twice from ethyl acetate and once from acetone: ether:petroleum (1:1:1) gave needle-shaped crystals having m.p. 162° (lit. 165°). Demethylation gave only galactose.

Fraction VI, (144 mg.) 2,3,4-tri-O-methyl-D-galactose.

R_G 0.70 in solvent A. $[\alpha]_D^{20}$ +111° (c, 1.0 in water).

This fraction when examined chromatographically was pure and corresponded to 2,3,4-tri-O-methyl-D-galactose in solvent A and C. On demethylation only galactose was obtained. It was shown to be 2,3,4-tri-O-methyl-D-galactose by conversion into

2,3,4-tri-O-methyl-N-phenyl-D-galactosylamine, m.p. 167° (lit. 167 - 170°)

Fraction VII, (236 mg.) 2,4-di-O-methyl-D-galactose.

R_G 0.53 (solvent A). $[\alpha]_D^{20}$ +106° ⟶ 89° (equil.) (c,1.0 in water).

Examination of this easily crystallised fraction in solvents A,B and C showed only one spot corresponding to standard 2,4-di-O-methyl-D-galactose. The crystalline sugar was further recrystallised from acetone to give colourless crystals melting at 97° which on admixture with authentic specimen of 2,4-di-O-methyl-D-galactose did not undergo depression. Demethylation gave only galactose.

Fraction VIII, (194 mg.) R_G 0.23 in solvent A.

This fraction was obtained on eluting the cellulose column with water. It gave a reddish-pink streak in solvent A and C. The syrup (50 mg.) was converted to the methyl ester methyl glycoside with dry methanolic hydrogen chloride, reduced with lithium aluminium hydride in dry tetrahydrofuran and hyrdolysed with 1N hydrochloric acid. Chromatographic examination of the product in solvents A and C showed only one spot (brown), R_G = 0.85, which corresponded to 2,3,4-tri-O-methyl-D-glucose. This was finally identified by the preparation of the aniline derivative (2,3,4-tri-O-methyl-N-phenyl-D-glucosylamine) which had m.p. and mixed m.p. 151°.

PERIODATE OXIDATION ON DEGRADED GUM

An aqueous solution (1%) of the whole gum was subjected to autohydrolysis at 100°C for 120 hours. The degraded gum was precipitated with ethanol, dialysed in running water for 3 days and freeze-dried. Yield ca. 52 mg. Hydrolysis with 2N sulphuric acidfor 6 hours ar 100°C and subsequent examination in solvents A, B and D showed that this degraded gum contained mainly D-galactose plus traces of L-arabinose, D-glucuronic acid, and 4-O-methyl-D-glucuronic acid.

40 mg. of this degraded gum was dissolved in water (5 ml.) and 1N sodium bicarbonate solution (5 ml.) was added to bring the pH to about 8. 1 ml. of 0.25M sodium metaperiodate solution was added and the reaction mixture was left in the dark at 20 ± 1°C. Aliquots (1 ml.) were withdrawn at varying intervals for the estimation of periodate uptake and for the estimation of the formaldehyde released. The former was estimated by diluting the aliquot (1 ml.) 250 times and measuring the optical density of the resulting solution in a Unicam spectrophotometer at 222.5mμ (48), an equimolecular solution of sodium iodate being used as control. To the other aliquot (1 ml.), 12.6% sodium sulphite solution (1 ml.) was added and the unreacted polysaccharide precipitated by the addition of ethanol (5 ml.) in a small centrifuge tube which was left overhight at 20°C. 1 ml. of the sppernatent liquid was taken and chromotropic acid reagent (9 ml.) added; this mixture was heated for ½ hour at 100°C, cooled, 2.6% thiourea solution (2 ml.) added, and the optical density of the resulting solution measured at 570 mμ (115, 116). The amount

of formaldehyde present was then read off a standard curve prepared by the periodated oxidation of D-glucose (97). The results are shown in the table below:-

Time of oxidation(hr.)	1½	6	18	25	44
HCHO(mol.)/anhydro galactose unit	0.034	0.043	0.069	0.078	0.078
Reduction of periodate per anhydro galactose unit.	0.44	0.59	0.79	0.84	0.84

The amount of formaldehyde released corresponded to 1 mole per 13 anhydrogalactose units.

"Smith Degradation" on Degraded Gum

The degraded gum was obtained from autohydrolysis of the whole gum (5% solution) for 24 hours, precipitated with ethanol, dialysed in running water for 2 days and freeze-dried. The gum had U.A.A.= 10.2%, corresponding to an equivalent weight of 1725. Hydrolysis of a small amount of the degraded gum and subsequent chromatographic examination revealed the presence of galactose and arabinose (in the ratio 3:1) together with D-glucuronic and 4-O-methyl-D-glucuronic acids. The degraded gum (1.5 g.) was dissolved in water (100 ml.) and to it freshly prepared sodium metaperiodate solution (100 ml.; 0.25M) was added; the solution was made upto 250 ml. In another flask 100 ml. of the same periodate solution was diluted to 250 ml. with distilled water. In a third 100 ml. of 0.25M sodium iodate solution was diluted to 250 ml. All solutions were shaken and kept in the dark at room tempepature. Aliquots (1 ml.) were withdrawn at varying intervals of time diluted 2500 times and the optical density of the resulting solution measured in a Unicam spectrophotometer at 222.5 mμ (48).

Also aliquots (5 ml.) were withdrawn from the reaction mixture, treated with a few drops of ethylene glycol to destroy excess periodate and the formic acid present titrated with 0.01N sodium hydroxide using methyl red as indicator. From these results the consumption of periodate and the release of formic acid were calculated both per anhydro galactose and per equivalent of the gum acid. The results are tabulated below:

Time of oxidation (hr.)	1½	6	18	25	44
Reduction of periodate (mol.) per anhydro galactose unit:	0.22	0.26	0.27	0.28	0.28
Per equivalent:	2.36	2.82	2.88	2.95	2.98
HCOOH (mol.) per anhydro galactose unit:	0.50	0.63	0.65	0.68	0.68
HCOOH (mol.) per equivalent:	5.4	6.7	7.0	7.3	7.3

No further uptake of the oxidant was observed after 44 hours when the degraded gum consumed 2,98 moles of periodate with liberation of 7.3 moles of formic acid per equivalent of the gum. At this stage excess periodate was destroyed by addition of ethylene glycol and the reaction mixture dialysed against running water for 48 hours. The dialysate was concentrated to small volume and reduction to the poly-alcohol effected by the addition of potassium borohydride (1 g.); the mixture was kept at room temperature for 2 days. The solution was then treated with Amberlite resins IR-120 and IR - 45, and concentrated to a syrup methanol was then added and the solution taken to dryness. This was repeated several times in order to remove all borate ions.

The poly-alcohol thus obtained (0.44 g.) was dissolved in 1N sulphuric acid (25 ml.) and allowed to stand at room temperature for 6 hours. The hydrolysis was stopped by neutralisation with barium hydroxide and barium carbonate. The neutralised soltuion was filtered treated with Amberlite resin IR - 120, concentrated to small volume and poured into excess ethamol. The precipitated "Smith degraded" polysaccharide was washed several times with ethanol before finally being dissolved in water, dialysed against running water for one day and freeze-dried (0.123 g.) . The alcohol extract and washings were taken to dryness to give low molecular weight materials (0.188 g.). Chromatographic examination of the latter in solvent B followed by spraying with aniline oxalate showed only two spots: one pink spot corresponding to arabinose and one bright yellow spot believed to be glycollic aldehyde. However, chromotagraphic examination of the alcohol extract in solvent B and G and spraying with silver nitrate showed three spots corresponding to arabinose, erythritol (heavy spot), glycerol and a fourth spot moving ahead of glycerol, believed to be glycollic aldehyde.

A portion (25 mg.) of the "Smith degraded" polysaccharide C on total hydrolysis with 2N sulphuric acid at 100°C furnished mainly galactose (heavy spot) plus faint spot corresponding to arabinose in solvents B, C and D.

"Smith degraded" polysaccharide C was subjected to periodate oxidation. The degraded polysaccharide C (60 mg.) was dissolved in water (10 ml.), 2 ml. of 0.25M sodium metaperiodate solution added and the mixture kept in the dark at room temperature for three days, after which time it was observed that 0.76 mole of periodate was reduced with the liberation of 0.33 mole of formic

acid per anhydro galactose unit.

D I S C U S S I O N

Structural studied on A. nilotica revealed that although this gum is broadly similar to other Acacia gums in main structural features, there were some significant differences as regards the acid residues. For structural studies the sample of A. nilotica selected was SS 62a. The main methods of investigation were partial acid hydrolysis (including autohydrolysis), methylation, and periodate oxidation (including "Smith degradation"). A summary of the work carried out on the gum from A. nilotica is given below.

A. NILOTICA

Comparative analytical studies on ten samples

(a) moisture, ash, nitrogen contents

(b) U.A.A. and OMe contents

(c) Viscosity measurements

(d) Optical rotation, (pH, F.R.S etc.).

(e) Equivalent weights

(f) Total hydrolysis and molar propotions of neutral sugars.

(g) Heterogeneity studies on DEAE-cellulose.

Structural studies on SS 62a

(a) autohydrolysis:-

3-O-β-L-arabinopyranosyl-L-arabinose

(b) Partial acid hydrolysis:-

6-O-β-D-galactopyranosyl-D-galactose

3-O-β-D-galactopyranosyl-D-galactose

6-O-(β-D-glucopyranosyluronic acid)-D-galactose

6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose

4-O-(α-D-glucopyranosyluronic acid)-D-galactose

4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose

(c) Methylation of the Whole Gum:-

2,3,5-tri-O-methyl-L-arabinose

2,5-di-O-methyl-L-arabinose

2,3,4-tri-O-methyl-L-arabinose

2,3-di-O-methyl-L-arabinose

2,3,4,6-tetra-O-methyl-D-galactose

2,4,6-tri-O-methyl-D-galactose

2,3,4-tri-O-methyl-D-galactose

2,4-di-O-methyl-D-galactose

2,3,4-tri-O-methyl-D-glucuronic acid

(d) Periodate oxidation ("Smith degradation")

Erythritol, glycerol, glycollic aldehyde + monosaccharides.

A. nilotica gum is completely soluble in water to give a pale yellow solution having $[\alpha]_D^{18}$ +108°. The apparent equivalent weight of the gum is 1894. Complete hydrolysis of the gum with 2N sulphuric acid showed the presence of D-galactose (44 %), L-arabinose (46 %), D-glucuronic acid and 4-O-methyl-D-glucuronic acid. A small amount of rhamnose (0.4%) was detected spectrophotometrically.

Autohydrolysis resulted in removal of most of the arabinose residues, leaving degraded gum B, composed essentially of galactose, D-glucuronic and 4-O-methyl-D-glucuronic acid, with some residual arabinose. Prolonged autohydrolysis (120 hours) did not result in cleavage of all the arabinose, and it therefore seems likely that the relatively resistant "core" of the gum molecule contains some arabinose.

The only disaccharide isolated at this stage was identified as 3-O-β-L-arabinopyranosyl-L-arabinose by the preparation of its crystalline phenylosazone.

This disaccharide gave only arabinose on hydrolysis. Methylation followed by hydrolysis of the disaccharide, gave 2,4-di-O-methyl-L-arabinose and 2,3,4-tri-O-methyl-L-arabinose. These were identified by paper chromatography as well as by the preparation of crystalline derivatives. The above, plus physical evidence($[\alpha]_D$ + 199°), indicated that the disaccharide was 3-O-β-L-arabinopyranosyl-L-arabinose . This disaccharide has been isolated from several other Acacia gums (see part I).

Partial Acid Hydrolysis

Hydrolysis of degraded gum B with 0.5N sulphuric acid gave a mixture of disaccharides and monosaccharides. Two neutral galactose-containing disaccharides were isolated by fractionation on thick paper in solvent B. The first fraction contained a disaccharide which crystallised from aqueous ethanol to give needles of m.p. and mixed m.p. 154°. Only galactose was obtained on hydrolysis with 1N sulphuric acid for 6 hours. Periodate oxidation resulted in the consumption of 1.94 mol. of oxidant per mol. disaccharide. Methylation, followed by hydrolysis, gave 2,3,4,6-tetra-O-methyl-D-galactose and 2,4,6-tri-O-methyl-D-galactose, proving the structure to be 3-O-β-D-galactopyranosyl-D-galactose. This was the major fraction and had $[\alpha]_D$ +57°.

The second disaccharide had $[\alpha]_D$ +28° and was chromatographically homogeneous. It had the same chromatographic mobility as authentic 6-O-β-D-galactopyranosyl-D-galactose in several solvents. Hydrolysis gave only galactose.

Hydrolysis of the whole gum with 1N sulphuric acid gave a mixture of monosaccharides and acid disaccharides. The neutral monosaccharides liberated were galactose and arabinose. The acid

fraction was separated from the neutral fractions on an anion-exchange resin (Duolite A-4), and was then fractionated on a cellulose column to give five fractions. The first fraction contained D-glucurone which on recrystallisation from water had m.p. and mixed m.p. 177°. The second fraction contained 4-O-methyl-D-glucuronic acid which was characterised as the amide of the methyl ester methyl glycoside. Furthermore reduction with potassium borohydride and hydrolysis furnished 4-O-methyl-D-glucose which was finally identified by the preparation of its phenylosazone. The third fraction contained mainly D-glucuronic acid while the last two fractions contained two acid disaccharides mixtures A and B. Each mixture was separated into two distinct aldobiouronic acids on thick paper in solvent D. The individual aldobiouronic acids were characterised as follows:

Acid mixture A on methylation, reduction with lithium aluminium hydride, and hydrolysis furnished three methylated sugars. These were separated on a cellulose column, and were identified as 2,3,4-tri-O-methyl-D-glucose, 2,3,4-tri-O-methyl-D-galactose and 2,3,6-tri-O-methyl-D-galactose through the preparation of crystalline derivatives. Fractionation of mixture A on thick paper gave two aldobiouronic acids:

(a) 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose. $[\alpha]_D^{20}$ +93°.

Hydrolysis gave only galactose and 4-O-methyl-D-glucuronic acid. Methanolysis and reduction with potassium borohydride, followed by hydrolysis, gave only galactose and 4-O-methyl-D-glucose; the latter must have arisen from the reduction of 4-O-methyl-D-glucuronic acid. Periodate oxidation resulted in the reduction of 1.8 moles of the oxidant per mole of methyl ester

methyl glycoside; the formation of formaldehyde indicated that C_6 was not involved in the linkage.

(b) 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose. $[\alpha]_D$ +6°.

Again hydrolysis gave only galactose and 4-O-methyl-D-glucuronic acid. Methanolysis followed by reduction with potassium borohydride and hydrolysis furnished galactose and 4-O-methyl-D-glucose. Furthermore the disaccharide consumed 3.1 moles of periodate on oxidation with 0.015M sodium meta periodate for 2 days. No formaldehyde was liberated. This foregoing evidence (including that of the methylation of of mixture A) suggests that the two aldobiouronic acids in mixture A were:

4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose

and 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose.

The last fraction (mixture B) on fractionation on thick paper in solvent D furnished two aldobiouronic acids. The first of these acids ($[\alpha]_D$ -5°) had the same chromatographic mobility as authentic 6-O-(β-D-glucopyranosyluronic acid)-D-galactose in several solvents. Hydrolysis furnished only galactose and D-glucuronic acid. Reduction of the derived methyl ester methyl glycoside with potassium borohydride, followed by hydrolysis, gave galactose and glucose only; the latter must have arisen from the reduction of D-glucuronic acid. The aldobiouronic acid was methylated, reduced with lithium aluminium hydride, and on hydrolysis furnished two methylated sugars identified chromatographically and by preparation of aniline derivatives as

2,3,4-tri-O-methyl-D-galactose and

2,3,4-tri-O-methyl-D-glucose.

This evidence indicates the aldobiouronic acid is 6-O-(β-D-gluco-

pyranosyluronic acid)-D-galactose, which has been isolated from all Acacia gums studied previously (see Part I).

The second component of mixture B gave galactose and D-glucuronic acid only on hydrolysis. The high ~~optical~~ specific rotation (+107°) indicated an α-linkage. Periodate oxidation resulted in a definite release of formaldehyde indicating that C_6 was not involved in the linkage. The aldobiouronic acid was believed to be 4-O-(α-D-glucopyranosyluronic acid)-D-galactose.

The 1,4-linkage was postulated by analogy with the aldobiouronic acid isolated earlier from A. karroo (75) which had similar optical rotation.

Methylation of Whole and Degraded Gum

Both the whole and degraded gum were methylated by Haworth's method and the methylation completed by Purdies method. The mixtures of methylated sugars obtained upon hydrolysis of the methylated polysaccharides were fractionated by partition chromatography on cellulose columns and the following sugars were characterised by formation of crystalline derivatives and/or by crystallisation of the sugars, as well as by gas chromatography of their methyl glycosides:

	whole gum Approx.wt.	Degraded gum Approx. wt.
2,3,5-tri-O-methyl-L-arabinose	41	14
2,5-di-O-methyl-L-arabinose	96	110
2,3-di-O-methyl-L-arabinose	21	trace
2,3,4-tri-O-methyl-L-arabinose	91	trace
2,3,4,6-tetra-O-methyl-D-galactose	196	64
2,4,6-tri-O-methyl-D-galactose	208	97
2,4-di-O-methyl-D-galactose	801	236
2,3,4-tri-O-methyl-D-galactose	157	144
2,3,4-tri-O-methyl-D-glucuronic acid		

Periodate Oxidation

Degraded gum B (obtained by auto-hydrolysis of the whole gum for 24 hours) was subjected to periodate oxidation and it was observed that after 44 hours the gum consumed 7,3 moles of periodate with the liberation of 2.98 moles of formic acid per equivalent (ca. 1725). The gum was subjected to "Smith degradation", and mild hydrolysis of the poly-alcohol with 1N sulphuric acid at room temperature furnished arabinose, erythritol, glycerol and glycollic aldehyde. The "Smith degraded"gum on total hydrolysis was observed to give mainly galactose plus a trace of arabinose. This gum was subjected to another periodate oxidation and was observed to consume 0.76 mole of periodate with the liberationof 0.33 mole of formic acid per anhydro galactose.

Degraded gum C (obtained by autohydrolysis of whole gum for 120 hours and found to contain mainly galactose) was also subjected to periodate oxidation and was found that, after 44 hours, 0.84 mole of periodate was consumed and 0.078 mole of formaldehyde liberated per anhydro galactose.

Structural Features of the Gum

The structural features of A. nilotica gum, on the basis of the foregoing results, can now be discussed.

Hydrolysis of the gum revealed the presence of residues of D-galactose, L-arabinose, D-glucuronic acid and 4-O-methyl-D-glucuronic acid. Autohydrolysis of the gum resulted in the liberation of labile sugar residues which have been identified as L-arabinose, and 3-O-β-L-arabinopyranosyl-L-arabinose, a disaccharide which has been obtained by careful hydrolysis of gum arabic (117) and of the gums from A. seyal (79) and A. karroo (75). Prolonged autohydrolysis of A. nilotica gum also produces some galactose. The characterisation of 3-O-β-L-arabinopyranosyl-L-arabinose as an autohydrolysis product of the whole gum establishes the presence of adjacent L-arabinose residues in the gum, as well as the fact that some of the L-arabinose end groups are in the pyranose form. This was further confirmed by the presence of 2,3,4-tri-O-methyl-L-arabinose in the hydrolysis products of the methylated gum.

As in the case of other Acacia gums so far investigated, mild hydrolysis removes a large proportion of the arabinose content but only a trace of galactose. It is therefore probable that these are in the outer parts of the molecule, and that many of the arabinose residues are in the furanosyl form. This has been confirmed by methylation studies on the whole gum which resulted in the isolation of a large proportion of 2,3,5-tri-O-methyl-L-arabinose. Furthermore, mild hydrolysis of the "Smith degraded" gum resulted in the liberation of L-arabinose which apparently occurs mostly in the furanosyl form since very little

2,3,4-tri-O-methyl-L-arabinose was present in the hydrolysis products of the methylated degraded gum. Also the most plentiful methylated arabinose present in the hydrolysis products of the methylated degraded gum was found to be a dimethylated arabinose, namely2,5-di-O-methyl-L-arabinose. It is probable that these units (together with the 2,3-di-O-methyl-L-arabinose isolated from the hydrolysis products of the methylated whole gum) originated from non-terminal arabinose residues in the outer chains, which are also resistant to attack by periodate, since the degradative procedure would remove the outer shell of arabinofuranose end- groups.

Careful hydrolysis of the whole gum resulted in the liberation of some galactose. Also both the methylated whole gum and degraded gum on hydrolysis furnished 2,3,4,6-tetra-O-methyl-D-galactose. This must also be a terminal group which is obviously in the pyranose form. Thus, taking into account the above results, the following moieties (mostly acid labile groups) exist in the gum:-

L-Araf1 ———

———5L-Araf1 ———

——— 4 L-Arap1 ———

L-Arap1 —β—3 L-Araf

D-Galp1———

However in view of the high arabinose content of the gum (46%) the existence of other disaccharides containing only arabinose (furanosyl form) cannot be ruled out. In particular the methylation data points to the existence of the disaccharide:

L-Araf1 ———5L-Araf

which might be isolated by very mild acid hydrolysis at room temperature.

Since there was very little galactose, and no galactose containing disaccharide, liberated during the autohydrolysis of the whole gum, it may be assumed that the "back-bone" of the gum is mainly made up of galactose residues. The isolation of 1,3- and 1,6-linked galactobioses from the partial acid hydrolysate of the degraded gum suggests that the galactan framework is made up of a highly branched system of 1,3- and 1,6-linked galactopyranose units. Since, however, the proportion of 1,3-linked galactose units is much higher than that of 1,6-linked units, it would appear that the main chain is made up of 1,3-linked galactose units and that the 1,6-linked galactose units are attached as side chains. This is supported by the fact that periodate oxidation of the degraded gum resulted in the consumption of 0.68 mole of periodate per anhydro-galactose.

The acidic fraction obtained as a result of the hydrolysis of the gum with 1N sulphuric acid has been found to contain four aldobiouronic acids, namely:

6-O-(β -D-glucopyranosyluronic acid)-D-galactose

6-O-(4-O-methyl- β -D-glucopyranosyluronic acid)-D-galactose

4-O-(α -D-glucopyranosyluronic acid)-D-galactose

and 4-O-(4-O-methyl- α -D-glucopyranosyluronic acid)-D-galactose

This is the first Acacia gum to yield more than two aldobiouronic acids. All the above four aldobiouronic acids have been isolated in pure form upon partial acid hydrolysis of A. nilotica gum. The first of these aldobiouronic acid is given by all the Acacia gums onpartial acid hydrolysis, while the third of these aldobiouronic acid has been isolated from A. karroo (75). The

remaining two aldobiouronic acids had not been isolated before from an Acacia gum. These acidic residues may occur in the labile side chains in the gum, as in most other Acacia gums. However, the possibility exists that the galactose residues in these aldobiouronic acids (particularly the last two acids) form part of the main chain.

Partial acid hydrolysis revealed that the following residues exist in the gum:

D-Galp1—β—3 D-Gal

D-Galp1 —β—6 D-Gal

and D-GluA1—β—6 D-Gal

4-O-MeD-GluA1—β—6 D-Gal

D-GluA1—α—4 D-Gal

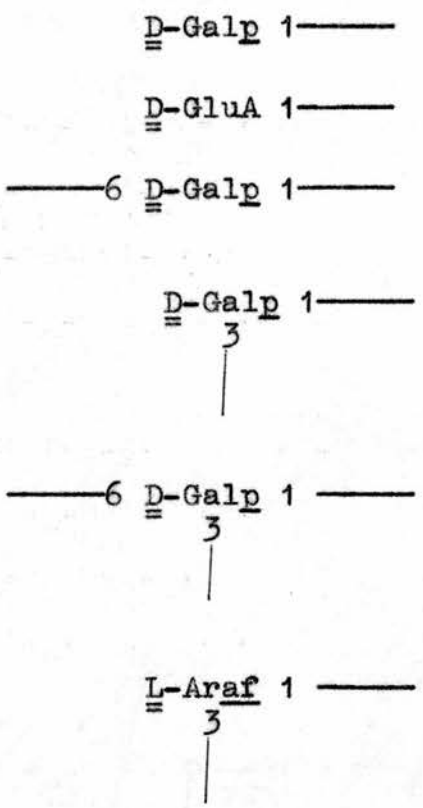
4-O-Me D-GluA1—α—4 D-Gal

Assuming that the methylation of the degraded gum was complete, the locations of the hydroxyl groups in the six main cleavage fraction indicate the positions through which the monosaccharide units are involved in union with other residues. Thus the isolation of 2,3,4-tri-O-methyl-D-glucuronic acid and 2,3,4,6-tetra-O-methyl-D-galactose demonstrates that these residues constitute end groups in the average repeating unit of the molecular complex since they are linked only through the reducing group at C_1. Since the molar proportions of these residues are approximately 2 to 1, it follows that there are twice as many glucuronic acid end groups as D-galactose end groups. Similarly the isolation of 2,3,4-tri-O-methyl-D-galactose shows that this component arises from D-galactose residues linked to other units through hydroxyl

groups at C_1 and C_6. These galactose residues which give rise to 2,4-di-O-methyl-D-galactose must be combined with other monosaccharide units through C_1 , C_3 , and C_6 and these are the main points of branching in the molecule. Since 2,4-di-O-methyl-D-galactose is the most plentiful product isolated from the hydrolysis products of the methylated gum, it follows that the gum molecule is highly branched.

Also the isolation of 2,4,6-tri-O-methyl-D-galactose shows that this residue arises from D-galactose residues linked through C_1 and C_3 in the original gum. The sixth main cleavage fraction of the methylated degraded gum is 2,5-di-methyl-L-arabinose and this may represent arabinose residues (furanosyl form) which are interposed between the terminal groups of the side chain and the nucleuses of the degraded acid. These may also be part of the nucleus itself; if so, the molecule of degraded gum may be composed of "blocks" of degraded gum units joined to each other possibly by arabofuranose units through C_1 and C_3. This suggestion is supported by the fact that not all the arabinose is removed by mild hydrolysis, however prolonged; furthermore "Smith degraded" gum still furnishes some arabinose. Such a degradative procedure should normally remove all the arabofuranose residues. It can be observed that the same methylated sugars occur in the hydrolysis products of the methylated degraded gum and whole gum, but, as expected, their relative proportions are different.

Considering the above results, the main residues as revealed by the methylation method, in the degraded gum are:-



It follows, from the methylation studies on the gum and from the isolation of 1,3 and 1,6 galactobioses on partial hydrolysis of the degraded gum, that the degraded gum contains a highly branched framework of 1:3- and 1:6-linked D-galactopyranose residues. Furthermore, the isolation of four aldobiouronic acids from A. nilotica gum, indicates that these residues must be important features of the gum molecule, but there is insufficient evidence at present regarding their location in relation to the galactan framework. Thus A. nilotica gum, which has the highest positive rotation encountered in any Acacia gum studied to date, reveals, on structural investigation, the following structural units:-

(A) End-groups:-

a. Neutral: L-Araf 1——

L-Arap 1 ——

D-Galp 1——

b. Acidic:

D-GluA 1——

or (4-O-Me-D-GluA ——)

(B) Disaccharides isolated from partial acid hydrolysis:-

a. Neutral:

L-Arap1—β— 3 L-Araf

D-Galp 1—β—3 D-Galp

D-Galp 1—β—6 D-Galp

b. Acidic:

D-GluA1—β—6 D-Galp

4-O-Me D-GluA 1—β—6 D-Galp

D-GluA 1—α—4 D-Galp

4-O-Me-D-GluA1 —α—4 D-Galp

(C) Non-terminal groups:-

a. Internal chain residues:

——3 L-Araf 1 ——

——4 L-Arap 1——

——5 L-Araf 1 ——

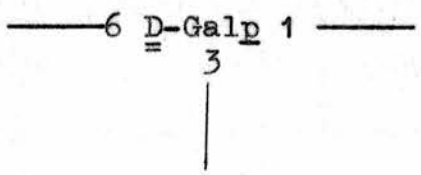
——6 D-Galp 1 ——

D-Galp 1 ——

3

|

b. Branching point:



It is impossible, with the results available as yet, to put forward a unique molecular structure for A. nilotica gum. Although a tentative structure for the degraded gum can be advanced — viz. a main chain of galactose residues linked 1,3 or 1,6 with side chains containing residues of D-galactose and D-glucuronic acid (or 4-O-Me-D-glucuronic acid)— it is not possible to decide, with the results available, whether the main chain linkages are 1:3 or 1:6, or perhaps some of each type. More work needs to be done to obtain further evidence on this point. In particular, information regarding the nature of the backbone of the gum molecule may be obtained by carrying out a series of "Smith degradations" on the whole gum.

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The analytical importance of the methoxyl content of *Acacia* gum exudates

SIR,

Methoxyl groups occur frequently in plant gums as 4-methoxyglucuronic acid (*e.g.*, in *Albizzia*[1] and *Khaya*[2] species) or as ester groups (*e.g.*, in *Sterculia*[3] and *Astragalus*[4] gums). To date, however, the possibility of the presence of methoxyl groups in *Acacia* gum exudates appears largely to have been ignored, *e.g.*, in studies of *A. senegal*,[5] *A. pycnantha*,[6] *A. karroo*,[7] *A. cyanophylla*[8] and *A. sundra*,[9] although Stephen reported (without comment) a value of 0·35% for *A. mollissima*,[10] and Hulyalkar *et al.* found no methoxyl content in *A. catechu*.[11]

Recently, Anderson and Herbich observed[12] that the methoxyl content of a number of nodules of the gum from *A. seyal* ranged from 0·7–1·3%, and this has led us to analyse specimens of the gum from 12 further *Acacia* species, not hitherto studied chemically. We have also re-investigated three different specimens of *A. senegal syn. Verek* (gum arabic) and a sample of *A. karroo*. An infrared method,[13] specific for methoxyl groups, was used to analyse purified samples, prepared from authenticated single nodules of each species by electrodialysis so that artifacts arising from solvent retention[14] could not occur (*cf.* ref. 1).

The results shown in Table I indicate that the presence of methoxyl groups in *Acacia* gums is a more general occurrence than hitherto believed. In addition, the range of values found (0·75 to 1·44%) for 9 nodules of *A. nilotica* substantiates recent evidence[12,15] for inter-nodule variation in the composition of plant gums.

The viscosity of samples of gum tragacanth and of pectins is known[16] to be related to their methoxyl content. The limiting flow-time numbers for some of our *Acacia* samples, determined under standardised conditions, are also shown in the Table: a plot of methoxyl content *versus* limiting flow-time number gives a smooth curve.

TABLE I

Acacia species	Methoxyl, %*	Limiting flow-time number†
A. giraffae Burch	2·40	
A. nilotica (L.) Willd. ex Del.	1·14[a]	10·4
A. mellifera (Vahl) Benth.	1·06	
A. seyal Del.	1·02[b]	12·1
A. seyal Del. var. fistula	0·90	
A. arabica (Lam) *Willd.*	0·88	12·5
A. tortilis (Forsk.) *Hayne.*	0·57	
A. mearnsii De Wild.	0·45	
A. campylacantha Hochst. ex A. Rich.	0·42	16·0
A. drepanolobium Harms ex Sjöstedt.	0·40	16·6
A. senegal (L.) *Willd.*	0·36[c]	19·2
A. dealbata Link.	0·35	21·5
A. laeta R. Br. ex Benth.	0·33	23·0
A. nubica Benth.	0·15	
A. karroo Hayne.	0·13	

* Electro-dialysed, freeze-dried samples, corrected for trace residual moisture and ash content.
† In aq. 4% NaCl solution at 25°.
[a] Average of results for 9 nodules (range 0·75–1·44%).
[b] Average of results for 6 nodules (range 0·70–1·30%).
[c] Average of results for 3 nodules (range 0·34–0·37%).

It is therefore suggested that the methoxyl content of *Acacia* gums has some structural significance, and that greater analytical attention should be given to this in future studies. We do not subscribe to the view, recently expressed[17] in a study of gum Jeol, that a methoxyl content of 0·51% can readily be dismissed as being very low and not structurally significant.

It is of interest that our re-examination of *A. senegal* and *A. karroo* has revealed the presence of methoxyl groups. Re-examination of other species, *e.g.*, *A. pycnantha*, may well provide an explanation for the complex behaviour observed[6] during examination of the aldobiuronic acid fraction.

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